

6

THE NEUTRAL DIVERGENCE OF QUANTITATIVE TRAITS

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In the preceding chapter, we learned how the opposing forces of random genetic drift and mutation lead to an equilibrium level of within-population genetic variance. In contrast, the phenotypic variance among isolated populations may continue to increase nearly indefinitely for neutral characters, as isolated demes or species recurrently acquire and become fixed for independent mutations. In the following pages, we explore the factors that drive the evolutionary dynamics of the among-population variance.

As in the preceding chapter, we will start with the situation in which the time span is short enough that most of the temporal dynamics for the change in population-mean phenotypes is driven by drift rather than by mutation. We then explore the consequences of longer-term divergence, with mutation playing an increasingly dominant role, showing that eventually the rate of divergence for neutral characters may become essentially independent of local effective population sizes.

Throughout, a central goal is to show how observed changes in the variance among populations can be used to test hypotheses regarding evolutionary mechanisms. Although few quantitative traits may actually evolve in a purely neutral fashion, a more compelling case for selection can always be made if the hypothesis of neutrality can be formally rejected. For example, an observed divergence of isolated lines that is significantly less than the neutral expectation provides evidence of stabilizing selection, whereas the reverse supports a role for diversifying selection. In addition, as populations become diminishingly small in size, drift begins to overwhelm selection, promoting nearly neutral patterns of evolution.

SHORT-TERM DIVERGENCE

We start with the special case in which all gene action is additive and random genetic drift is the only evolutionary mechanism. Most of the predictions of this model can be expressed in terms of two observable quantities: the additive genetic variance in the base population, and the effective population sizes of the isolated lineages. The expected among-population genetic variance, $\sigma_B^2(t)$, under neutrality is obtained by noting that the mean genotypic value at a diallelic locus i is $2a_i p_i$ (there being two genes per locus, each with additive effect a_i with probability p_i , and effect 0 with probability $1 - p_i$). The variance among populations for this locus is then $E\{[2a_i p_i(t)]^2\} - \{E[2a_i p_i(t)]\}^2$, which simplifies to $4a_i^2 \sigma_{p_i}^2(t)$, where $\sigma_{p_i}^2(t)$ is the expected among-population variance in allele frequency. Summing over all loci, assuming negligible gametic-phase disequilibrium, and substituting from Equation 2.12a,

$$\begin{aligned} \sigma_B^2(t) &= 4 \sum_{i=1}^n a_i^2 p_i(0) [1 - p_i(0)] \left\{ \frac{1}{N_f} + \left[1 - \left(1 - \frac{1}{2N_e} \right)^t \right] \right\} \\ &= \left(\frac{1}{N_f} + 2f_t \right) \sigma_A^2(0) \end{aligned} \quad (6.1)$$

where N_f is the effective number of founders per line, and the time index is defined such that $t = 0$ denotes the final generation of the base population and $t = 1$ denotes the founder generation for the isolated lines. Equation 6.1 shows that, under the assumptions of the ideal model, the expected variance among genotypic means of isolated populations increases linearly with the inbreeding coefficient, asymptotically approaching a limit very close to twice the additive genetic variance in the base population (Wright 1951).

Ignoring the generally minor contribution from the baseline founder effect, this limiting result may be obtained in a simpler manner. Because the probability of fixation of a neutral allele is equal to its initial frequency, when the process of random drift is allowed to go to completion, a proportion $p_i(0)$ of the populations will have genotypic value $2a_i$, and a proportion $1 - p_i(0)$ will have genotypic value 0. The mean genotypic value is therefore $2a_i p_i(0)$ and the mean squared value is $4a_i^2 p_i(0)$, which yields the among-population variance $4a_i^2 p_i(0) [1 - p_i(0)] = 2\sigma_{A_i}^2(0)$. Under the assumption of additivity, the lower version of Equation 6.1 holds regardless of the number of alleles at the underlying loci.

It should be noted that the preceding expression considers only the true genetic divergence among lines, which can in principle be obtained by an analysis of variance of phenotypic variation within and among lines after factoring out the former from the estimated mean squares. If however, one simply focuses on the raw variance of the observed means, additional sources of variation, associated with finite sample sizes, will contribute to the observed divergence (Hill 1972; Lynch 1988). For example, when the mean phenotype of each line is determined from n progeny from $N/2$ matings (involving $N/2$ males and females, for a total parental sample size of N), there would be three additional sources of variance to add to Equation 6.1: 1) segregational variance resulting from the sampling of $Nn/2$ individuals, $(1 - f_{t-1})\sigma_A^2(0)/(Nn)$ (the segregational variance in the population being equal to half the total variance); 2) variance associated with maternal effects resulting from the sampling of $N/2$ mothers $\sigma_{E_g}^2/(N/2)$; and 3) residual variance associated with

special environmental effects averaged over the entire progeny pool, $\sigma_{E_s}^2/(Nn/2)$. In addition, assuming no transmission of maternal effects across generations, the among-line variances in consecutive generations will be correlated as a consequence of inheritance,

$$\sigma_B(t, t') = \left(\frac{1}{N_f} + 2f_t \right) \sigma_A^2(0) \quad \text{for } 0 < t < t' \quad (6.2)$$

A few words should also be said about the potential importance of nonadditive gene action. From Table 5.2, it can be seen that in the presence of dominance, the among-population variance eventually asymptotes at $\sigma_B^2 = 2\sigma_A^2 + 2\sigma_{ADI} + \sigma_{DI}^2$. Thus, dominance can magnify or reduce the among-population variance depending upon the magnitudes of σ_{DI}^2 and σ_{ADI} and on the sign of the latter. In addition, the asymptotic contribution from epistatic interactions involving additive effects is equal to $2^n \sigma_{A^n}^2$ for n -locus epistasis, i.e., $4\sigma_{AA}^2$ for additive \times additive epistasis, and $8\sigma_{AAA}^2$ for additive \times additive \times additive epistasis. Thus, epistasis involving large numbers of loci can, in principle, greatly magnify the development of among-population variance, even if it appears to be of relatively minor importance within populations.

Sampling Error

We now consider the sampling properties of the among-population genetic variance by reference to a particular type of experiment, again assuming a character with a strictly additive basis (Hill 1972; Lynch 1988). Starting from a base population with additive genetic variance $\sigma_A^2(0)$, L replicate lines are isolated and subsequently maintained each generation with $N/2$ random monogamous matings. Due to the fact that only a finite number of lines is studied, the among-population variance that actually develops in any particular experiment, $\hat{\sigma}_B^2(t)$, will deviate from the expectation $\sigma_B^2(t)$. Moreover, due to finite sample sizes within populations, the among-population variance estimated by the investigator, $\text{Var}(B, t)$, will deviate from $\hat{\sigma}_B^2(t)$. The first source of variation, the evolutionary variance, is a function of population-genetic structure and, for a fixed system of mating, is largely beyond the control of the investigator. The second source of variation, the sampling variance, can be minimized by the use of large sample sizes in deriving parameter estimates.

Our concern here is primarily with the variation in the realized outcome of the divergence process resulting from the stochastic nature of random genetic drift, so again we focus on the situation in which the among-line divergence has been measured in such a way as to eliminate nongenetic causes. Suppose that the same experiment has been repeated many different times, on each occasion starting with L lines from the same base population. Due to the variation in the drift process and the finite number of observed lines, each set of experimental lines will develop its own temporal pattern of realized among-population variance. The expected variation in the realized variance among these hypothetical replicate experiments provides a measure of confidence that one can have in the results of any single experiment. Letting $\hat{\sigma}_B^2(t)$ be the realized among-population variance at generation t for a particular

experiment, the expected variance of this quantity among replicate experiments is

$$\sigma^2[\hat{\sigma}_B^2(t)] \simeq \frac{4\sigma_A^4(0)}{L-1} \left[\frac{1}{2N_f^2} + 2 \left(1 + \frac{1}{N_f} \right) f_t^2 + \sigma_f^2(t) \right] \quad (6.3)$$

Although, in practice, one generally performs such a divergence experiment only once, the utility of this function is that it is entirely in terms of observable parameters, so that some idea of the reliability of estimates of $\sigma_B^2(t)$ can be determined in advance.

In most situations, the terms in Equation 6.3 involving the founder number (N_f) will be of second or third order and can be ignored. The term $\sigma_f^2(t)$ has been examined in detail in Lynch (1988), drawing heavily from the results of Weir et al. (1980) and Cockerham and Weir (1983). For freely recombining loci, σ_f^2 is zero when the pedigree structure is fixed, e.g., obligate selfing, full-sib mating, the maximum avoidance systems of Wright (1921), and the circular systems of Kimura and Crow (1963); and even with fairly tightly linked loci, $\sigma_f^2(t)$ is generally negligible in any generation under selfing or full-sib mating. However, under most natural mating schemes, some individuals mate by chance with closer relatives than do others. This results in variation in f among members of the same population, which because of sampling, accumulates as among-population variance in f . The theoretical value of $\sigma_f^2(t)$ under different systems of mating is of special interest because empirical studies usually do not record the essential pedigree information for its computation. For larger population sizes, even with unlinked loci, if the sexes are separate and matings are monogamous, the squared coefficient of variation $[\text{CV}(f_t)]^2 = \sigma_f^2(t)/f_t^2$ can attain values of 0.1 to 1.0 in the first two to four generations of isolation, which is enough to contribute significantly to $\sigma^2[\hat{\sigma}_B^2(t)]$. However, after six or so generations have passed, $\sigma_f^2(t)$ can be safely ignored regardless of the population size, even with tightly linked loci.

These results indicate that ignoring the initial founder effect, the coefficient of variation of the among-population variance is equivalent to $\sqrt{2\{1 + [\text{CV}(f_t)]^2\}/(L-1)}$, which is generally on the order of $\sqrt{2/L}$, in some cases being as high as $2/\sqrt{L}$. Thus, studies of phenotypic divergence need to be very large to be statistically reliable. For example, if it is desirable to reduce the standard error of the among-line variance to 10% of the expectation under the null hypothesis of neutrality and additivity, a minimum of 200 lines should be studied.

One way to achieve fuller clarity on the genetic mechanisms driving short-term divergence is to focus on the temporal pattern of increase in σ_B^2 , which as noted above, should eventually reach a constant as $f \rightarrow 1$. When a temporal sequence of estimates of the among-population variance is available, these may be regressed on f_t . Under the null hypothesis of neutral additive genes, the expected slope of such a regression is $2\sigma_A^2(0)$. However, because of inheritance, consecutive estimates of mean phenotypes obtained from the same lines are nonindependent, violating a fundamental assumption of least-squares regression analysis. For example, once the lines have become completely inbred, all future values of $\hat{\sigma}_B^2(t)$ must be fixed, and therefore should not be given equal weight in the regression analysis. The expected covariance of $\hat{\sigma}_B^2$ between generations with inbreeding levels f_t and $f_{t'}$ is

$$\sigma[\hat{\sigma}_B^2(t), \hat{\sigma}_B^2(t')] \simeq \frac{4\sigma_A^4(0)}{L-1} \left[\frac{1}{2N_f^2} + 2 \left(1 + \frac{1}{N_f} \right) f_t f_{t'} + \lambda_1^{t'-t} \sigma_f^2(t) \right] \quad \text{for } t < t' \quad (6.4)$$

Lynch (1988) provides approximate expressions for the standard errors of the slope and intercept that account for the intrinsic correlations in the data, assuming measurements of σ_B^2 in progressive generations. The variance of the regression coefficient increases with the duration of the experiment, but is essentially constant after the fourth generation of inbreeding. At that point, the standard error ranges from approximately $4\sigma_A^2(0)/\sqrt{L}$ under obligate self-fertilization to $3\sigma_A^2(0)/\sqrt{L}$ with larger N_e , implying coefficients of variation in the range of $1.5/(f\sqrt{L})$ to $2/f\sqrt{L}$, which for large f , are not greatly different from the sampling variances of single-point estimates noted above.

Empirical Observations

As an example of the application of the preceding formulae, consider the results from a large drift experiment with laboratory cultures of the flour beetle *Tribolium castaneum* (Rich et al. 1984). The authors followed twelve replicate populations at four population sizes (1:1 sex ratio, random mating) over 20 consecutive generations. Each generation, the mean pupal weight (in μg) of each population was obtained from a bulk sample of 100 random individuals. The additive genetic variance was estimated to be 460 in the base population. The observed $\text{Var}(B, t)$ are plotted as a function of f_t in Figure 6.1, along with the expected divergence $920f_t$ (solid lines). The dashed lines, obtained by use of Equation 6.3 under the assumption of a χ^2 distribution with 11 degrees of freedom, give the limits of the among-population variance beyond which there is less than a 5% chance for the realization of the drift process in either direction. Since these bounds ignore measurement error, they may be regarded as conservative confidence limits. Nevertheless, almost all of the observations, with the exception of the clusters of the late generations at $N = 10$ and 20, lie within these limits. The least-squares regressions of the data are given by the dotted lines. The slope of each regression is less than the expected 920, but all are within two standard errors of the expectation. Thus, even in the absence of a control, the observed patterns are fairly consistent with a hypothesis of random drift of neutral additive genes. The observed declines in $\text{Var}(B, t)$ late in the experiment at the two smallest population sizes may have simply arisen by chance and remained there due to intergenerational correlations.

–Insert Figure 6.1 Here–

The results of some other short-term divergence experiments given in Figure 5.3 exhibit no evidence for nonlinear increases in the among-population variance with inbreeding. Eisen and Hanrahan (1974) have argued that the divergence of growth and reproductive rates in inbred lines of mice is more rapid than can be accounted for by the additive genetic variance in the base population, and Bryant et al. (1986) suggested the same for morphological traits in bottlenecked housefly lines. In neither case, however, was it verified that the departures from expectations were significant.

Example 6.1. For the case in which one has only a single estimate of the among-population divergence, $\text{Var}(\bar{z}, t)$, Lande (1977) suggested the statistic

$$F = \frac{\text{Var}(\bar{z}, t)}{t \cdot \text{Var}(A, 0)/N_e} \quad (6.5)$$

as a test for neutrality. The denominator of this expression is an estimate of the expected among-population variance, which is obtained from $2f_t\sigma_A^2(0) = 2\{1 - [1 - 1/(2N_e)]^t\}\sigma_A^2(0) \simeq t\sigma_A^2(0)/N_e$ for $t \ll N_e$. Under the assumption of a normal sampling distribution of population mean phenotypes, F is expected to be F -distributed. This requires that the numerator be χ^2 -distributed with variance equal to $2[2f_t\sigma_A^2(0)]^2/(L-1)$. Ignoring the added contribution from sampling error, this can be seen to be approximately true for large N by reference to Equation 6.3. However, with selfing and full-sib mating, the expected variance is about twice and 1.5 times too high respectively. Thus, Lande's approach should be restricted to lines with at least moderate effective size. Moreover, as we will see below, all of the formulae in this section become questionable for $t > N_e$, because they ignore the contribution from new mutations.

Lande (1977) used Equation 6.5 to evaluate the results of a 12-year divergence experiment involving five populations of *Drosophila pseudoobscura* (Anderson 1973). Two of the populations had been maintained at 25°C, two at 27°C, and one at 16°C. They were then raised in two common environments (16 and 25°C) and measured for wing length. Estimates of the additive genetic variance for these two environments were 0.88 and 0.77, while the among-population variances were approximately 6.62 and 4.37 respectively. An approximate upper bound for the number of generations of divergence is $t = 150$, whereas the effective population size probably always exceeded $N_e = 1000$. The use of these extreme bounds gives conservative estimates of F , making it more difficult to demonstrate diversifying selection on wing length. Even so, the ratios of observed to expected among-population variance are 50 and 38, both of which are highly significant (comparing these with the critical F ratio with four degrees of freedom in the numerator, and infinite degrees of freedom in the denominator). Thus, the hypothesis that the observed line divergence is solely attributable to random genetic drift can be rejected confidently. More likely, the different thermal conditions resulted in selection for different wing lengths.

LONG-TERM DIVERGENCE

Polygenic mutation was first incorporated into the theory of population divergence by Dempster (appendix in Bailey 1959) and was subsequently studied by Lande (1976), Chakraborty and Nei (1982), and Lynch and Hill (1986). Again focusing on a character with a purely additive genetic basis, starting with an ancestral-population genetic variance of $\sigma_A^2(0)$, and assuming the infinite-alleles model, the expected variance of genotypic means for replicate populations isolated t generations in the past is

$$\sigma_B^2(t) = 2\sigma_m^2 t + 2[\sigma_A^2(0) - 2N_e\sigma_m^2][1 - e^{-t/(2N_e)}] \quad (6.6)$$

where σ_m^2 is the per-generation mutational rate of input of genetic variance, as described in Chapter 5. This expression shows that as t becomes large, the expected

rate of increase of the among-population variance for a neutral quantitative trait becomes a constant $2\sigma_m^2$ per generation. The same formulation applies to the among-species genetic covariance for a pair of traits, if the mutational rate of production of covariance between the traits is substituted for σ_m^2 (Lande 1979).

Thus, under the infinite-alleles model, the asymptotic divergence rate is independent of the population size, just as it is in the neutral theory of molecular evolution (Kimura 1983). Although the expected number of new mutations entering a population each generation is $2Nu$ per locus, the probability of fixation of a new mutation is its initial frequency $1/(2N)$, so the expected number of mutations fixed per locus per population per generation is simply u . For a set of L populations, with each fixed mutation causing an increase in expected among-population variance of $\sim E[(2a)^2]$, and n loci contributing, the asymptotic divergence rate is $nuLE[(2a)^2/L] = 2\sigma_m^2$.

Under the assumptions of the infinite-alleles model, the asymptotic divergence rate of $2\sigma_m^2$ is a fairly general result. It is independent of the degree of dominance of new mutations, of the linkage relationships of the constituent loci, and of the mating system (Lynch and Hill 1986). This is because both dominance and gametic-phase disequilibrium are transient properties of alleles en route to loss or fixation, and not cumulative phenomena, and because the problem of fixation of a new neutral mutation is equal to its initial frequency regardless of the breeding system.

How long should populations be isolated before one should start to worry about the contribution of new mutations to their divergence? From Equation 6.6, it can be seen that this depends on the initial level of genetic variance and on the effective sizes of the derived isolates. In Figure 6.2 it is assumed that the initial base population is in drift-mutation equilibrium, so that $\sigma_A^2(0) = 2N_e\sigma_m^2$, and that the isolated lineages have rapidly attained the same effective sizes (N_e). Under these circumstances, by the time N_e generations have elapsed, polygenic mutation subsequent to the isolation event has caused about 20% of the divergence, whereas once $t > N_e$ generations, essentially all of the divergence is due to new mutations.

–Insert Figure 6.2 Here–

As emphasized in the preceding chapter, alternatives exist to the infinite-alleles model, raising questions about the appropriate structure of a neutral null model. For example, Cockerham and Tachida's (1987) model, which assumes a finite number of alleles with each new mutational effect being independent of the prior allelic state (the house-of-cards model), yields an equilibrium among-population variance

$$\sigma_B^2 = 2[1 - E(H)]\sigma_A^2(\infty) \quad (6.7)$$

where from Chapter 5, $E(H)$ is the expected heterozygosity per locus, and $\sigma_A^2(\infty) = 2nE(a^2)$ is the expected additive genetic variance in a population of infinite size. Note that under this model, not only does the among-population variance not build up indefinitely, but as $4N_eu \rightarrow \infty$, driving the heterozygosity to 1.0, the among-population component of variance asymptotically approaches zero. This is because under the house-of-cards model, replicate populations that are each effectively infinite in size

will individually harbor the same alleles with the same frequency spectrum defined by the mutational interconversion rates.

If nothing else, these dichotomous results indicate that although neutral models are essential to demonstrating the necessity of invoking natural selection to explain an observed pattern of divergence, the actual construction of the null model depends on unresolved biological issues. Using the bridge model developed by Zeng and Cockerham (1993), discussed in Chapter 5, the equilibrium among-population variance becomes

$$\sigma_B^2 = \frac{4E(a^2)}{(1-\tau)^2[1+4N_e u(1-\tau)]} \quad (6.8)$$

where $\tau = 1$ under the Lynch-Hill model and 0 under the Cockerham-Tachida model. For $\tau < 1$, the approach to the equilibrium level of divergence is defined by the mutation rate (u), assuming an identical N_e in the base and descendant populations,

$$\sigma_B^2(t) = [1 - (1-u)^{2t}] \sigma_B^2 \quad (6.9)$$

and hence quite slow (approximately $2u$ per generation).

Finally, we note that the expression for the variance of the among-population variance (i.e., the variance of $\sigma_B^2(t)$ among replicate experiments) is algebraically complex, and has only been worked out for the infinite-alleles model (Lynch and Hill 1986). However, if it is assumed that the number of loci is large and the distribution of mutational effects is normal with mean zero, the variance of the realized among-population variance approaches $2(2\sigma_m^2 t)^2/L$ for large t . This is simply twice the square of the expected among-population variance. Thus, for large t , the coefficient of variation of a realized among-population variance based on L lines is expected to be on the order of $\sqrt{2/L}$, so as we have noted before, unless L is quite large, estimates of σ_B^2 can deviate quite far from the expectation.

Effectively Neutral Divergence and the Estimation of Rates of Mutational Variance

As discussed in LW Chapter 12 in detail, the theoretical expectations of the neutral model provide the basis for estimating the rates of polygenic mutation. Starting from an inbred base population, experimental lines with known times of divergence can be used to estimate the amount of polygenic mutation that is necessary to account for the distribution of the resultant mean phenotypes. In one of the earliest endeavors of this sort, Russell et al. (1963) started with several lines of maize that had been maintained by prolonged self-fertilization. They then performed a dichotomous branching experiment for five generations in which each plant was self-fertilized to produce two new daughter sublines. Seed was saved from each generation, so that at the end of the experiment members of all generations could be assayed simultaneously in a common environment, and then sib analysis was used to estimate the additive genetic variance for the *total* population each generation. Assuming the within-population variance to be in drift-mutation equilibrium, this type of population expansion should give rise to an average rate of increase in the total genetic variance of $2\sigma_m^2/\text{generation}$. In accordance with this prediction, the regressions of the genetic variance on time were positive for every character investigated (Figure

6.3). The rate of polygenic mutation for each of the traits is estimated by one-half the slope.

Results from many other experiments of this sort were reviewed in Lynch and Walsh (1998). Although a number of additional results have emerged since then, most of these are confined to a small number of model systems, and the conclusions reached in the earlier review remain unaltered. Here, we simply give a brief update, providing references only to post-1998 papers. Estimates of h_m^2 for a diversity of morphological, physiological, and life-history traits in *D. melanogaster* are consistently in the range of 0.001 to 0.005. Mutational heritabilities for body size and life-history traits in nematodes fall in the range of 0.001 to 0.008 (Vassilieva et al. 2000; Baer et al. 2006; Ostrow et al. 2007), and the same is true for life-history traits in the microcrustacean *Daphnia pulex* and in the grape phylloxera *Daktulosphaira vitifoliae* (Downie 2003). Thus, essentially all studies with invertebrates imply $0.001 < h_m^2 < 0.01$ for complex traits.

Although the numbers of studies are still rather limited, estimates of h_m^2 for some land plants and vertebrates appear to be several-fold higher than those noted above. Mutational heritabilities for growth and reproductive traits in *Arabidopsis thaliana* are in the range of 0.001 to 0.008 (Schultz et al. 1999; Shaw et al. 2000; Chang and Shaw 2003; Kavanaugh and Shaw 2005), but the average h_m^2 for maize, from the study of Russell et al. (1963), is 0.0092. In addition, mutational heritabilities for morphological and reproductive traits in mice fall in the range of 0.003 to 0.023 (Casellas and Medrano 2008). Thus, there is at least a rough indication that mutational heritabilities are increased in organisms with longer life spans, which might in principle be a consequence of elevated rates of mutation per generation (Chapter 4).

Finally, it should be emphasized that in all mutation-accumulation experiments, fitness declines in the vast majority of lines, indicating that mutations are on average deleterious, although the fraction of mutations that are beneficial remains unclear (Shaw et al. 2002; Keightley and Lynch 2003; Charlesworth and Eyre-Walker 2007; Eyre-Walker and Keightley 2007; Dickinson 2008; Hall et al. 2008). Equally importantly, for characters that influence fitness only indirectly (e.g., morphology), the fraction with negative pleiotropic effects on fitness remains unclear. Resolving this issue is critical to any attempts to utilize estimates of mutational heritability to infer long-term mechanisms of evolution, as illustrated in the following section.

–Insert Figure 6.3 Here–

TESTING THE NULL HYPOTHESIS OF NEUTRAL PHENOTYPIC DIVERGENCE

One of the enduring problems in all areas of evolutionary biology is the struggle to demonstrate that various aspects of biodiversity are products of diversifying selection. It is one thing to concoct plausible adaptive scenarios to explain patterns of morphological, physiological, or behavioral divergence, but quite another to formally demonstrate that the possibility that an observed level of divergence cannot

simply be explained by a null model of random genetic drift. The preceding theory suggests a number of ways in which this might be done.

Long-term Drift of Mean Phenotypes Among Isolated Species

One of the first approaches to testing the null model of long-term neutral divergence of mean phenotypes was suggested by Lande (1976). As noted above, the expected variance among mean phenotypes after t generations of divergence under the infinite-alleles model is $2\sigma_m^2 t$. However, although this expression may be of direct use if an accurate estimate of σ_m^2 exists, such estimates are available for only a moderate number of traits in a few species. An alternative approach is to recall that the equilibrium heritability under the neutral model is $h^2 = 2N_e\sigma_m^2/\sigma_z^2$, where σ_z^2 is the within-population phenotypic variance for the trait. Thus, for populations assumed to be in equilibrium, the expected among-population (species) variance for a neutral character can also be written as

$$\sigma_B^2 = h^2\sigma_z^2 t/N_e \quad (6.10)$$

The utility of this expression is that all of the parameters are potentially observable.

Assuming a character with a multifactorial basis, following a neutral phase of drift and mutation, the deviation of the mean phenotype of an isolated population from that of the base population is expected to be approximately normally distributed with mean zero and variance σ_B^2 defined in Equation 6.6. The absolute change in mean phenotypes, $|\Delta\bar{z}|$, is then expected to exceed $1.96\sigma_B(t)$ with probability 0.05. Rearranging Equation 6.10, we arrive at a critical effective population size below which there is a $> 5\%$ probability of a deviation as large as an observed magnitude $\Delta\bar{z}$,

$$N_e^* = \frac{(1.96)^2 h^2 t}{(\Delta\bar{z}/\sigma_z)^2} \quad (6.11)$$

If the true effective population size were known to exceed N_e^* , then one would reject the neutral hypothesis with 95% confidence. Alternative arrangements of Equation 6.11 can be made to determine critical divergence times or critical heritabilities for a given amount of divergence, and substitution of 2.58 for 1.96 raises the statistical bar to the 99% confidence level. For example, substituting $2N_e\sigma_m^2/\sigma_z^2$ for h^2 , Equation 6.11 can be rewritten as

$$(\sigma_m^2)^* = \frac{(\Delta\bar{z})^2}{2t(1.96)^2} \quad (6.12)$$

where $(\sigma_m^2)^*$ denotes the minimal rate of production of mutational variance for the trait that is compatible with the observed level of divergence (Turelli et al. 1988). Thus, if estimates of σ_m^2 and t are available, one can test the neutral hypothesis without an estimate of N_e , and in this case if σ_m^2 were known to be $< (\sigma_m^2)^*$, one would have to invoke diversifying selection to explain the observed level of divergence.

For any analysis of this sort to be meaningful, one must be confident that the magnitude of $\Delta\bar{z}$ is not inflated by environmental effects on phenotypes, which is clearly problematical when populations cannot be assayed in a “common-garden environment”. In addition, the divergence of means ought to be estimated by an

analysis of variance so as to eliminate the inflation of the divergence by sampling error of the within-population means. Combining these considerations with the fact that the previous expressions for critical effective population sizes or critical mutation rates ignore the sampling error of all other terms on the right, and that the infinite-alleles model for neutral evolution may be too extreme, it should be clear that the preceding tests for neutrality cannot be regarded as very rigorous in a statistical sense, and are best employed as diagnostic guides for future study.

Finally, although the preceding theory has focused on the issue of excess divergence relative to the neutral expectation, one can also inquire as to whether the *stability* of population means is too great to be compatible with neutrality. In a two-tailed test for neutrality, the critical 2.5% probability cutoff for $|\Delta\bar{z}|/\sigma_B(t)$ being too small to be consistent with the model is 0.03, whereas the critical 2.5% cutoff for excessively high divergence is 2.24, so that by substituting these two numbers into Equations 6.11 and 6.12, one can test the joint hypothesis as to whether the observed level of divergence is too high or too low for neutrality (at the total probability level of 0.05).

Numerous attempts have been made to apply the above procedures, or simple variants of them, to data from the fossil record to test the hypothesis that levels of morphological divergence over geological time scales have been driven by directional selection. For example, in the first of such studies, Lande (1976) showed that change in tooth-size dimensions over a 42 million year period in early horse evolution are consistent with the hypothesis of random genetic drift if the heritabilities of the traits had been near 0.5 and the long-term effective population size was smaller than 60,000 or so individuals. Given the generally high levels of heritability observed for mammalian morphological traits (Lynch and Walsh 1998), an assumption of $h^2 = 0.5$ is not unreasonable, and the argument that the long-term N_e in such lineages could be smaller than the critical value $N_e^* = 60,000$ is also plausible (Chapter 4), which would imply that drift could have acted alone to cause the observed changes. Analyses of tooth morphometrics in two additional lineages of extinct mammals (condylarths and oreodonts) suggest critical effective sizes of 80,000 to 120,000 below which the observed changes would be compatible with a neutral hypothesis. Thus, only if the effective sizes of these ancient mammalian taxa were actually in excess of 10^5 , a matter that remains unclear, would the observed changes require some mechanism of directional selection.

Several other studies of this nature have been applied to aspects of mammalian skull evolution. For example, by taking the upper and lower limits to mutational heritability, σ_m^2/σ_e^2 , to be 10^{-2} and 10^{-4} , Lynch (1990) found that the rates of evolution of cranial morphology in a wide array of placental mammalian lineages are one to two orders of magnitude below the minimum neutral rate, and Lemos et al. (2001) observed a similar pattern in marsupials. The only exception to this general trend concerns the races of modern man, which appear to have diverged at a rate slightly above the minimum neutral expectation (Lynch 1990; Ackermann and Cheverud 2004; Roseman 2004). Although they leave many questions unanswered, these kinds of results put in perspective previous arguments that rates of morphological evolution are exceptionally high in mammals, and especially so in the great apes (e.g., Cherry et al. 1982; Wyles et al. 1983; Van Valen 1985). Clearly, the predominant mode of evolution in mammalian skeletal morphology has been one of stabilizing

selection, not of strong diversifying selection.

These types of comparative morphological studies, which are subject to many of the problems outlined above (most notably the potential influence of the environment on mean phenotypes), can now be extended to the molecular level. It is now possible, for example, to measure the level of expression for essentially the full repertoire of an individual's genes using **microarray analyses**, which quantify relative transcript levels from the intensity of hybridization of **cDNAs** (reverse transcripts of messenger RNA) to microdeposits of complementary probes on a small wafer. The numerous technical aspects of microarray analysis are glossed over here, the main point being that, with appropriate controls, it is possible to isolate the genetic component of gene-expression variance among individuals and sometimes between closely related species.

The general conclusion from such work is that stabilizing selection plays a prominent role in reducing levels of genetic variance in gene expression below the neutral expectation – both within and among species, levels of variation are much lower than expected based on presumed levels of mutational variance. For example, using lines of the nematode *C. elegans* from a long-term (280 generations) mutation-accumulation experiment, Denver et al. (2005) estimated σ_m^2 for several thousand genes. By comparing levels of variation among a global collection of natural isolates, they found that ratios of standing levels of genetic variance to σ_m^2 were generally no greater than a few hundred. Given that this ratio provides an estimate of $4N_e$ under the assumption of neutrality in a selfing organism (as opposed to $2N_e$ in an outcrosser), these observations provide a firm rejection of the hypothesis that gene expression levels evolve in a neutral fashion. Rifkin et al. (2005) were able to estimate mutational heritabilities for mutation-accumulation lines of *D. melanogaster* by factoring out the variance at the individual fly level to obtain an estimate of σ_e^2 . They found a median $h_m^2 \simeq 2.4 \times 10^{-5}$ across all genes, and showed that although interspecific variance in the expression of a gene was correlated with its mutational variance (in qualitative accordance with the neutral theory), the absolute level of divergence was too low to be compatible with neutrality (consistent with the results from Denver et al. 2005).

This conclusion of strong stabilizing selection on gene expression appears to extend to mammals, despite the fact that the efficiency of selection would be expected to be reduced as a consequence of low effective population sizes (Chapter 4). For example, Lemos et al. (2005) found that levels of gene-expression variance among intraspecific strains of mice average about two orders of magnitude below the minimum neutral expectation, whereas those between mouse species and between human and chimpanzee are eight to ten orders of magnitude too low for neutrality. Evaluating primates more broadly (human, chimpanzee, orangutan, and rhesus macaque), Gilad et al. (2006) found that the among-species variance in expression of most genes did not increase with divergence time, contrary to the neutral expectation; this study was particularly nicely designed in that it employed only DNAs for which the sequences were identical across all four species.

Quite contrary to the preceding interpretation, Khaitovich et al. (2004, 2005) have argued that gene expression in the great apes evolves in a largely neutral fashion. However, their arguments are based on observations that are only loosely connected with neutral expectations: a positive correlation between levels of within-

and among-species variation for the expression of different genes; and a linear increase in among-species expression divergence with time. Because the genetic components of within- and between-species variance are both driven by mutation, they are indeed expected to be correlated under the neutral model. However, because gene expression is a function of both the genetic and environmental background of an individual, unless the latter is factored out in a quantitative-genetic analysis, such measures provide uncertain information on the more relevant levels of genetic divergence. Genes whose expression is strongly influenced by the environment may naturally exhibit higher levels of variation both within and among samples. In addition, unless the actual rate of divergence is consistent with the rate of polygenic mutation, linear patterns of evolutionary diversification need not imply neutrality, and may instead be a consequence of random fluctuating selection. Thus, the conclusion that primate gene expression is evolving in a neutral fashion appears to be questionable, and has in fact been essentially retracted by in a more recent analysis (Chaix et al. 2008), which suggests an elevation in rate specific to the human lineage.

Taken together, these results suggest, perhaps not surprisingly, that at both the phenotypic and gene-regulatory levels, mammalian evolution is primarily characterized by periods of stabilizing selection, although relatively brief episodes of directional selection cannot be ruled out. However, it must also be emphasized that the interpretation of conservative rates of evolution is far from clearcut. In principle, evolutionary divergence rates that are below the expectation of the Lynch-Hill model may be a consequence of the general opposition of selection to all allelic changes associated with the trait, but there might also simply be a fraction of mutations that is truly neutral and another that has strong negative pleiotropic effects on fitness. In that case, an observed level of divergence could actually be entirely based on neutral mutations, but with the appropriate measure of mutational variance being lower than the actual value observed in mutation-accumulation experiments (where even highly deleterious mutations can accumulate). Alternatively, if the Cockerham-Tachida model is a more appropriate model, then one would expect cumulative levels of divergence to plateau in time rather than to increase indefinitely, not because of direct selective constraints but because of limited availability of alternative allelic states.

Finally, it is worth noting that the methodology outlined above has primarily been used to test the neutral hypothesis with two-point analyses (i.e., with phenotypic measures at two points in time in a vertical lineage, or from two extant species derived from a recent common ancestor). A temporal series of data provides a more powerful means of analysis, as it then becomes possible to look for statistical trends in mean phenotypes or for correlations in rates of change in adjacent intervals, neither of which are expected in a strictly neutral model (at least under the infinite-alleles model) (Charlesworth 1984; Bookstein 1988; Estes and Arnold 2006). Even in these kinds of studies, however, one must be concerned with the possibility that trends in the data are driven by environmental effects on trait expression rather than by underlying genetic changes.

Population Subdivision for Quantitative Traits

An alternative approach to testing the neutral hypothesis of divergence focuses on subpopulations of the same species, isolated by semipermeable migration barriers, as discussed in Chapter 3. By obtaining allele-frequency estimates for a diversity of neutral molecular markers from multiple subpopulations, one can partition the allelic diversity for the entire metapopulation (measured as heterozygosity under the assumption of panmixia) into its within- and among-subpopulation components (Cockerham 1973; Nei 1987; Weir 1996). The fraction of diversity associated with subpopulation divergence is generally called F_{ST} (or sometimes G_{ST}) in deference to Wright (1951), who first suggested this measurement of population subdivision.

Now consider a quantitative trait with a purely additive-genetic basis, and let Q_{ST} denote the level of population subdivision for allele frequencies at the loci underlying the trait. Letting the genetic variance for the trait in the entire metapopulation under the assumption of panmixia be σ_G^2 , then from the theory developed earlier in this chapter as well as in Chapter 5, the within- and among-subpopulation components of variance can be represented as $\sigma_{GW}^2 = (1 - Q_{ST})\sigma_G^2$ and $\sigma_{GB}^2 = 2Q_{ST}\sigma_G^2$, respectively. It follows that

$$Q_{ST} = \frac{\sigma_{GB}^2}{\sigma_{GB}^2 + 2\sigma_{GW}^2} \quad (6.13)$$

(Lande 1992; Spitze 1993). This is a very general result, applying to a wide range of population structures and migration patterns provided the character does indeed have an additive genetic basis (Whitlock 1999).

Equation 6.13 provides a potential empirical method for testing the hypothesis of neutral divergence among subpopulation means. If isolates of sufficient numbers of families from multiple subpopulations can be grown in a common environment, then appropriate statistical methods (Lynch and Walsh 1998) can be used to estimate σ_{GW}^2 and σ_{GB}^2 . The resultant estimate of Q_{ST} can then be compared to a parallel measure of subdivision (F_{ST}) derived from putatively neutral markers. Under the assumption of neutrality Q_{ST} should not be significantly different from F_{ST} . However, $Q_{ST} > F_{ST}$ is expected if subpopulation differentiation has been primarily driven by adaptive divergence, whereas the opposite pattern is expected if the mean phenotypes of all or most subpopulations are kept relatively uniform by stabilizing selection for the same optima. Whitlock (2008) provides a broad overview of the use of comparisons of Q_{ST} and F_{ST} in tests for selection, including an evaluation of the form of the distributions of both statistics.

Because of the requirement for assays in a common-garden arena, joint studies of Q_{ST} and F_{ST} are not common. However, the majority of results, over a diverse assemblage of animals and land plants, support a hypothesis of $Q_{ST} \simeq F_{ST}$ or $Q_{ST} > F_{ST}$ (Leinonen et al. 2008). As the latter results are qualitatively consistent with adaptive differentiation, they are clearly at variance with the observations on longer-term divergence noted above.

One major caveat with respect to this strategy for testing for neutral divergence is that, even under neutrality, the expected value of Q_{ST} will not necessarily equal F_{ST} if the trait of interest is influenced by nonadditive genetic effects. This is because, as outlined in Chapter 5 and above, with nonadditive gene action, the within- and among-subpopulation components of genetic variation for neutral characters under short-term divergence are no longer equal to $\sigma_{GW}^2 = (1 - f)\sigma_G^2$ and $\sigma_{GB}^2 = 2f\sigma_G^2$ (where f is the parameter estimated by F_{ST}), but instead are influenced by a num-

ber of higher-order terms. In general, because the within-population genetic variance declines less rapidly with inbreeding under nonadditivity (and sometimes even increases; Chapter 5), Q_{ST} as defined by Equation 6.13 will tend to be smaller than F_{ST} under neutrality, although exceptions do exist (Goudet and Büchi 2006; López-Fanjul et al. 2003, 2006, 2007). By encouraging the false impression of stabilizing selection, this general behavior makes conclusions regarding adaptive divergence based on elevated Q_{ST} conservative, while rendering observations of $Q_{ST} < F_{ST}$ ambiguous.

QTL Analysis of Divergent Lines

The preceding approaches rely on large samples of multiple populations or at two or more intervals. However, there is a situation in which one might test for adaptive divergence with just a single cross between two isolated lineages. By examining a battery of polymorphic markers segregating in the F_2 generation of such a cross, one may search for QTLs associated with phenotypic measures of various traits (Lynch and Walsh 1998). Under the neutral hypothesis, the relative abundances of “plus” and “minus” marker alleles associated with small vs. large phenotypes are expected to be randomly distributed should not differ significantly from a 1:1 ratio.

This general strategy will be biased if the parental lines are intentionally selected to have extreme phenotypes, as the high line would then naturally be expected to be enriched with “plus” alleles. However, Orr (1998) suggested a way around this problem. If enough QTLs have been identified so that their distribution of effects can be approximated, given the level of phenotypic divergence between the lines, one can computationally evaluate the probability that the observed number of “plus” alleles in the high line could have arisen by chance. Although Orr (1998) gives a few examples of the application of this method to some artificial systems, it has not been extensively applied, the main limitation being the development of a fairly accurate estimate of the distribution of QTL effects, which in turn requires a survey of high-density markers in a substantial number of F_2 individuals.

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Figure 6.1. Observed and expected levels of the among-population variance for pupal weight in a divergence experiment with the flour beetle *Tribolium*. The lines are described in the text. Data from Rich et al. (1984).

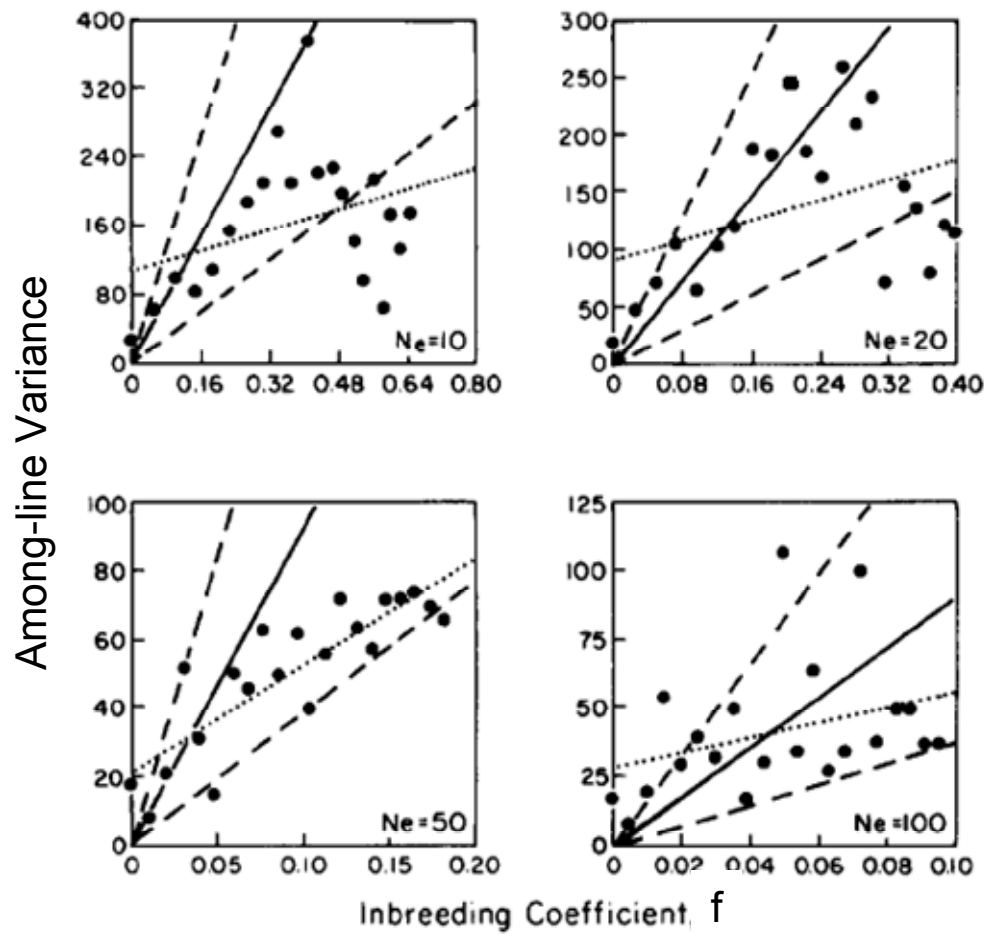


Figure 6.2. The expected fraction of among-population variance attributable to mutations arising subsequent to the isolation event. It is assumed that the base population is in drift-mutation equilibrium, $\sigma_A^2(0) = 2N_e\sigma_m^2$, with the same effective size as the daughter species, so that from Equation 6.6, the divergence due to base-population variance is $2\sigma_A^2(0)[1 - e^{-t/(2N_e)}]$. To obtain the actual number of generations of divergence for any population size, multiply the horizontal axis by N_e .

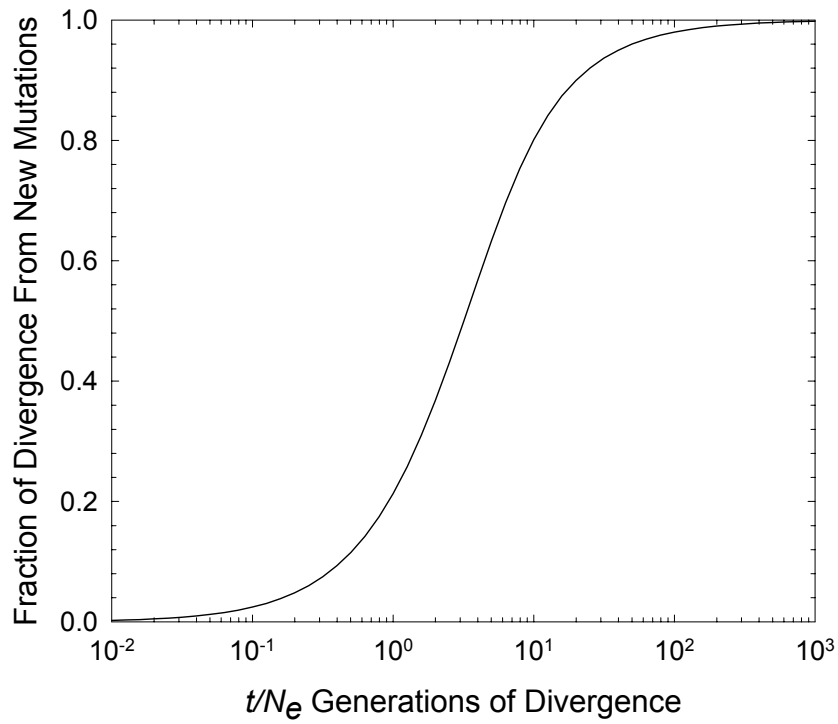


Figure 6.3. The increase in additive genetic variance (within- plus among-population components) in an expanding set of lines of corn. From Russell et al. (1963).

