

## POPULATION MIXING AND THE ADAPTIVE DIVERGENCE OF QUANTITATIVE TRAITS IN DISCRETE POPULATIONS: A THEORETICAL FRAMEWORK FOR EMPIRICAL TESTS

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**Abstract.**—Empirical tests for the importance of population mixing in constraining adaptive divergence have not been well grounded in theory for quantitative traits in spatially discrete populations. We develop quantitative-genetic models to examine the equilibrium difference between two populations that are experiencing different selective regimes and exchanging individuals. These models demonstrate that adaptive divergence is negatively correlated with the rate of population mixing ( $\hat{m}$ , most strongly so when  $\hat{m}$  is low), positively correlated with the difference in phenotypic optima between populations, and positively correlated with the amount of additive genetic variance ( $G$ , most strongly so when  $G$  is low). The approach to equilibrium is quite rapid (fewer than 50 generations for two populations to evolve 90% of the distance to equilibrium) when either heritability or mixing are not too low ( $h^2 > 0.2$  or  $\hat{m} > 0.05$ ). The theory can be used to aid empirical tests that: (1) compare observed divergence to that predicted using estimates of population mixing, additive genetic variance/covariance, and selection; (2) test for a negative correlation between population mixing and adaptive divergence across multiple independent population pairs; and (3) experimentally manipulate the rate of mixing. Application of the first two of these approaches to data from two well-studied natural systems suggests that population mixing has constrained adaptive divergence for color patterns in Lake Erie water snakes (*Nerodia sipedon*), but not for trophic traits in sympatric pairs of benthic and limnetic stickleback (*Gasterosteus aculeatus*). The theoretical framework we outline should provide an improved basis for future empirical tests of the role of population mixing in adaptive divergence.

**Key words.**—Adaptation, dispersal, gene flow, migration, natural selection, stickleback, water snake.

Received April 20, 2000. Accepted October 18, 2000.

Natural selection should lead to the adaptive divergence of populations in different environments, whereas the exchange of individuals (and their genes) should oppose that divergence. Theoretical models have confirmed that high levels of population mixing (or gene flow) can indeed constrain adaptive divergence (e.g., Haldane 1948; Slatkin 1973, 1978; Felsenstein 1976; Endler 1977; Barton and Gale 1993; García-Ramos and Kirkpatrick 1997), but the relative importance of population mixing in the wild remains a matter of debate (Ehrlich and Raven 1969; Slatkin 1987; Storfer 1999). In the absence of a clear consensus, empirical tests become increasingly important. Moreover, it is often of interest to determine if maladaptive traits within specific populations are the result of immigration from elsewhere (e.g., Stearns and Sage 1980; Dhondt et al. 1990; Riechert 1993; King and Lawson 1995; Storfer and Sih 1999). Despite the obvious importance of empirical work, tests for the role of population mixing have been surprisingly haphazard in certain contexts, particularly for quantitative traits in spatially discrete populations. We feel that this deficiency can be attributed to the lack of a clearly outlined theoretical framework on which to base such tests.

Our goal is to show how theory can be used as a guide for empirical tests. We first outline quantitative-genetic models for how selection and population mixing oppose each other during adaptive divergence. We then apply aspects of this theory to empirical data from two natural systems, demonstrating that population mixing constrains adaptive diver-

gence in island versus mainland water snakes (*Nerodia sipedon*), but not in benthic versus limnetic threespine stickleback (*Gasterosteus aculeatus*). A variety of terms have been used when discussing exchange between populations (e.g., migration, gene flow, dispersal), and no consistent standard has been adopted (Endler 1977; Neigel 1997). To avoid ambiguity, we use “population mixing” or “mixing” when referring to the movement of organisms, gametes, or propagules among discrete populations, and “gene flow” when referring to the resulting long-term exchange of genes.

### THEORETICAL FRAMEWORK

Theoretical developments and associated empirical tests of a role for mixing (or gene flow) in constraining adaptive divergence have been excellent in certain contexts, such as spatial clines in selection (e.g., Porter et al. 1997; Lenormand et al. 1998) and Mendelian traits in discrete populations (e.g., Goodisman et al. 2000). We focus on a context where theory has largely been absent: quantitative traits in spatially discrete populations. Our theoretical development is based on the consideration of differences in mean phenotype between two populations. The populations have different optimal trait values and are linked by some level of population mixing ( $\hat{m}$ , defined explicitly below). In our first model (MS), the populations mix and then selection acts in each. In our second model (SM), selection acts in each population and then they mix. MS might be most appropriate for organisms that disperse as eggs or juveniles, whereas SM might be most appropriate for organisms that disperse as adults. Although the models are illustrated for a two population scenario, the same framework could be used to develop models for more complex situations.

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### Mixing, Then Selection (MS)

Consider two populations with mean trait values of  $\bar{z}_i$  and  $\bar{z}_j$ , where subscripts  $i$  and  $j$  refer to the different populations. The mean trait value in population  $i$  after mixing between them ( $\bar{z}_i^m$ ) is  $\bar{z}_i^m = m_i\bar{z}_j + [1 - m_i]\bar{z}_i$ , where  $m_i$  is the proportion of population  $i$  made up of immigrants from population  $j$ . The mean trait value after mixing and then selection (i.e., mean breeding value,  $\bar{z}_i^{ms}$ ) is  $\bar{z}_i^{ms} = \bar{z}_i^m + G_i\beta_i(\bar{z}_i^m)$ , where  $G_i$  is the additive genetic variance for the trait and  $\beta_i$  is the selection gradient acting on the trait (as defined by Lande and Arnold 1983). Note that  $\beta_i$  is a function of  $\bar{z}_i^m$ , written  $\beta_i(\bar{z}_i^m)$ , because the strength of selection will depend on the mean trait value at any given time. The change in mean trait value in one generation ( $\Delta\bar{z}_i$ ) is  $\Delta\bar{z}_i = \bar{z}_i^{ms} - \bar{z}_i$  or, after substitution from the above,  $\Delta\bar{z}_i = m_i\bar{z}_j - m_i\bar{z}_i + G_i\beta_i(\bar{z}_i^m)$ . The subscripts are reversed for population  $j$  (i.e.,  $\bar{z}_j^m$ ,  $\bar{z}_j^{ms}$ , and  $\Delta\bar{z}_j$ ). We are interested in the difference in mean trait value between the populations ( $D = \bar{z}_j - \bar{z}_i$ ) and the change in this difference each generation ( $\Delta D = \Delta\bar{z}_j - \Delta\bar{z}_i$ ), which after substituting from the above is given by

$$\Delta D = m_j(\bar{z}_i - \bar{z}_j) + G_j\beta_j(\bar{z}_j^m) - m_i(\bar{z}_j - \bar{z}_i) - G_i\beta_i(\bar{z}_i^m). \quad (1)$$

We now define  $\hat{m} = m_i + m_j$ , which represents the total amount of population mixing ( $\hat{m}$  approaches unity as mixing approaches 100%). Using  $\hat{m}$ , equation (1) becomes

$$\Delta D = -\hat{m}D + G_j\beta_j(\bar{z}_j^m) - G_i\beta_i(\bar{z}_i^m). \quad (2)$$

The equilibrium difference in mean trait value between populations when mixing takes place before selection ( $D_{ms}^*$ ) is found by setting  $\Delta D = 0$  and solving for  $D$ , yielding

$$D_{ms}^* = \frac{1}{\hat{m}}[G_j\beta_j(\bar{z}_j^m) - G_i\beta_i(\bar{z}_i^m)]. \quad (3)$$

Phenotypic traits are often related to each other through genetic correlations and therefore respond to both direct and indirect selection (Lande and Arnold 1983). The equivalent solution for correlated traits is the same as equation (3), except that  $D_{ms}^*$  is replaced with a vector of equilibrium differences in mean trait values ( $\mathbf{D}_{ms}^*$ ),  $G_i$  and  $G_j$  are replaced with matrices of additive genetic variances/covariances for the traits ( $\mathbf{G}_i$  and  $\mathbf{G}_j$ ), and  $\beta_i$  and  $\beta_j$  are replaced with vectors of selection gradients acting on the traits ( $\boldsymbol{\beta}_i$  and  $\boldsymbol{\beta}_j$ ). Note that the strength of the selection gradients depends on the difference in optimal trait values between populations, but the optima themselves need not be known to predict the equilibrium difference (it is sufficient to measure selection in each population).

### Selection, Then Mixing (SM)

A similar analytical framework can be applied when selection takes place before mixing. The mean trait value in population  $i$  after selection (i.e., mean breeding value,  $\bar{z}_i^s$ ) is  $\bar{z}_i^s = \bar{z}_i + G_i\beta_i(\bar{z}_i)$ . The mean trait (breeding) value after mixing ( $\bar{z}_i^{sm}$ ) is  $\bar{z}_i^{sm} = m_i[\bar{z}_j + G_j\beta_j(\bar{z}_j)] + [1 - m_i][\bar{z}_i + G_i\beta_i(\bar{z}_i)]$ , and its change in one generation (i.e.,  $\Delta\bar{z}_i = \bar{z}_i^{sm} - \bar{z}_i$ ) is  $\Delta\bar{z}_i = m_i[\bar{z}_j - \bar{z}_i] + m_iG_j\beta_j(\bar{z}_j) + [1 - m_i]G_i\beta_i(\bar{z}_i)$ . (Reverse subscripts for population  $j$ .) Note that this equation for a single trait can be derived from Via and Lande's (1985) model when plasticity is ignored. In their equation A6, set  $\bar{z}_{11} = \bar{z}_{21}$ ,  $\bar{z}_{22}$

$= \bar{z}_{12}$ , and  $G_{ij} = G$ ; and in our equations set  $m_i = m(1 - q)$  and  $m_j = mq$ .

As before, we are primarily interested in the difference in mean trait value between populations, and the per generation change in that difference ( $\Delta D = \Delta\bar{z}_j - \Delta\bar{z}_i$ ), which after setting  $\hat{m} = m_i + m_j$  and substituting from the above becomes

$$\Delta D = -\hat{m}D + [1 - \hat{m}][G_j\beta_j(\bar{z}_j) - G_i\beta_i(\bar{z}_i)]. \quad (4)$$

The equilibrium difference when mixing takes place after selection ( $D_{sm}^*$ ) is therefore

$$D_{sm}^* = \frac{(1 - \hat{m})}{\hat{m}}[G_j\beta_j(\bar{z}_j) - G_i\beta_i(\bar{z}_i)]. \quad (5)$$

The corresponding equation for correlated traits is the same, except that each term is replaced with its vector or matrix equivalent. As in MS, the selection gradients depend on the difference in optimal trait values between populations, but the optima need not be known to predict the equilibrium difference.

### Equilibrium Differences under Stabilizing Selection in Each Population

To consider the influence of each parameter on the equilibrium difference between populations, particular forms must be specified for  $\beta_i$  and  $\beta_j$  (because they are functions of  $\bar{z}_i$  or  $\bar{z}_i^m$  and  $\bar{z}_j$  or  $\bar{z}_j^m$  and will therefore change when other parameters change). Assuming stabilizing selection around a phenotypic optimum in each population (resulting in a Gaussian fitness function; Via and Lande 1985), the selection gradient in population  $i$  can be expressed as

$$\beta_i = \frac{-[\bar{z}_i - \theta_i]}{\omega_i^2 + P_i}, \quad (6)$$

where  $\theta_i$  is the optimal trait value,  $\omega_i$  is the strength of stabilizing selection (width of the fitness function), and  $P_i$  is the phenotypic variance. When selection acts on a mixture (as in MS),  $\bar{z}_i$  in equation (6) is replaced with  $\bar{z}_i^m$  (i.e.,  $m_i\bar{z}_j + [1 - m_i]\bar{z}_i$ ). (Reverse subscripts for population  $j$ .)

Assuming  $\omega$ ,  $P$ , and  $G$  are the same in each population (for simplicity) and substituting the appropriate version of equation (6) for  $\beta_i(\bar{z}_i^m)$  and  $\beta_j(\bar{z}_j^m)$  in equation (3), the equilibrium difference in trait value when mixing occurs before selection (MS) is

$$D_{ms}^* = D_0 \left[ \frac{G}{G(1 - \hat{m}) + (\omega^2 + P)\hat{m}} \right], \quad (7)$$

where  $D_0$  is the difference in optimal trait value between the populations ( $\theta_j - \theta_i$ ). The equilibrium difference between populations when selection occurs before mixing (SM) is

$$D_{sm}^* = D_0 \left[ \frac{G(1 - \hat{m})}{G(1 - \hat{m}) + (\omega^2 + P)\hat{m}} \right]. \quad (8)$$

Equations (7) and (8) reveal several patterns. First, an increase in population mixing decreases the equilibrium difference in mean trait value between populations, and this effect is strongest when mixing is lowest (Fig. 1A). Second, the equilibrium difference increases linearly with increasing difference between the optima (Fig. 1B). Third, increasing

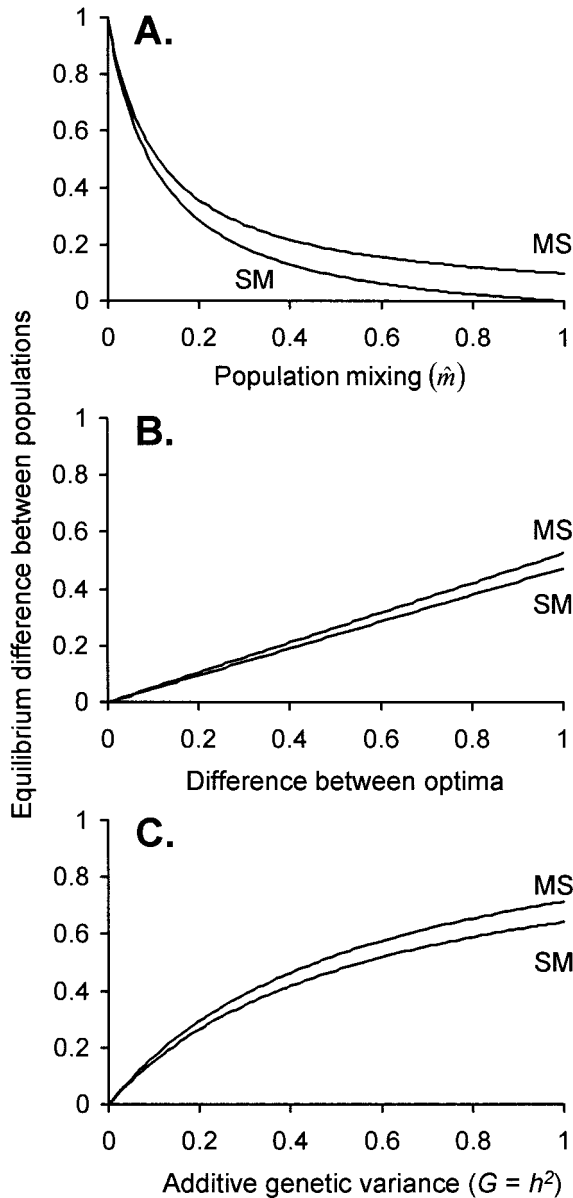


FIG. 1. The influence of variation in parameter values on the equilibrium difference in trait values when mixing takes place before selection (MS, using eq. 7) and when mixing takes place after selection (SM, using eq. 8). (A) As population mixing ( $\hat{m}$ ) increases, equilibrium differences decrease rapidly from the optimum and approach zero asymptotically. (B) As the difference between optima increases, equilibrium differences increase linearly. (C) As the amount of additive genetic variation increases, equilibrium differences increase toward an asymptote. Except for the parameter being varied in each case,  $D_0 = 1$ ,  $G = 0.5$ ,  $\hat{m} = 0.1$ ,  $P = 1$  (so that  $G$  equals the narrow-sense heritability,  $h^2$ ), and  $\omega = 2$  (so that the strength of stabilizing selection is weak relative to the phenotypic variance).

additive genetic variance increases the equilibrium difference (Fig. 1C). Fourth, the equilibrium difference is larger when mixing takes place before selection because the effect of immigrants is immediately reduced through natural selection in MS, but not in SM. Two-way interactions between mixing and genetic variance are shown in Figure 2.

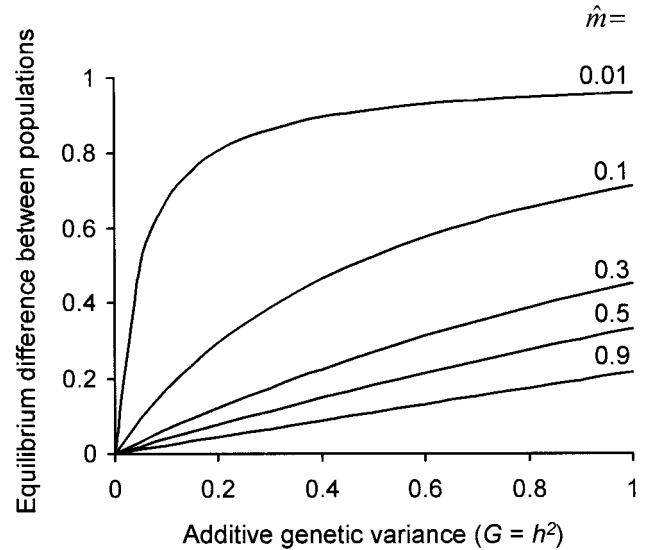


FIG. 2. Combined influences of population mixing ( $\hat{m}$ ) and additive genetic variance ( $G$ ) on the equilibrium difference in trait values when mixing takes place before selection (MS, using eq. 7). Similar patterns were obtained when mixing takes place after selection (SM, using eq. 8, not shown).  $P = 1.0$ ,  $\omega = 2.0$ ,  $D_0 = 1.0$ .

#### Rate of Approach to Equilibrium

Equations (3), (5), (7), and (8) predict differences in qualitative traits between populations under equilibrium conditions. This assumption means that we should be concerned about how long a perturbed system takes to reach equilibrium. To address this issue, we consider the length of time required for a pair of nonequilibrium populations to evolve 90% of the distance to equilibrium. For example, if two populations are currently identical, but the optimal difference between them is 1.0 standard deviation (SD), we consider the length of time required to reach a difference of 0.1 SD.

This analysis is illustrated for MS, but the final equation is identical for SM. We consider a single trait because results for the correlated-trait model depend heavily on nuances of  $\mathbf{G}$ , and the possible outcomes become unmanageable to present. We first incorporate the Gaussian stabilizing selection function (6) (using  $\bar{z}_i^m$  and  $\bar{z}_j^m$ ) into equation (2) and assume that  $\omega$ ,  $P$ , and  $G$  are the same in each population:

$$\Delta D = -\hat{m}D - G \frac{[D(1 - \hat{m}) - D_0]}{\omega^2 + P}. \quad (9)$$

To consider the dynamics of this relationship, we introduce a new variable ( $\tilde{D}$ ), which represents the amount by which the current difference deviates from the equilibrium difference (i.e.,  $D - D^*$ ). The change in  $\tilde{D}$  over time ( $\Delta \tilde{D}$ ) is given by

$$\Delta \tilde{D} = -\tilde{D} \left[ \frac{G(1 - \hat{m}) + (\omega^2 + P)\hat{m}}{\omega^2 + P} \right], \quad (10)$$

which can be solved for the deviation from equilibrium as a function of time,  $\tilde{D}(t)$ ,

$$\tilde{D}(t) = \tilde{D}(0) \left[ \left( 1 - \hat{m} \left( 1 - \frac{G}{\omega^2 + P} \right) \right)^t \right]. \quad (11)$$

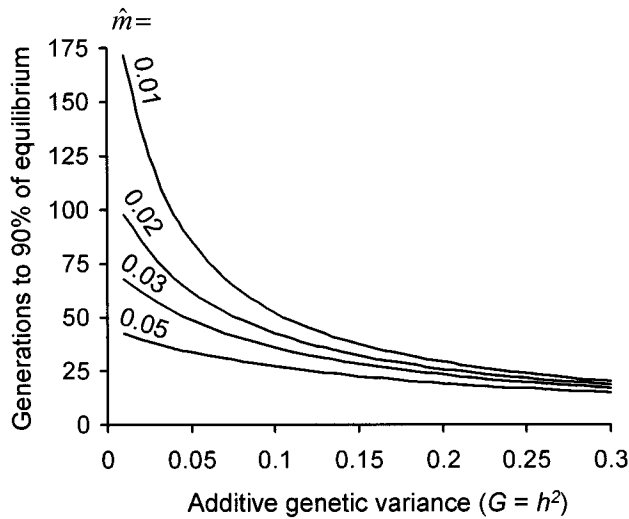


FIG. 3. Rate of approach to equilibrium as influenced by additive genetic variance and population mixing ( $\hat{m}$ ) in equation (13). Lines show the number of generations taken for the difference between two nonequilibrium populations to evolve 90% of the distance to equilibrium ( $P = 1.0$ ,  $\omega = 2.0$ ,  $k = 0.9$ ).

$\tilde{D}(0)$  is the initial deviation from equilibrium ( $t = 0$ ), and the rate of approach to equilibrium is given by the term raised to the  $t$ th power. Note that  $\tilde{D}(t)$  will approach zero as time goes to infinity and, because the term raised to the  $t$ th power is less than unity, the change will occur more quickly when the term is smaller. Now consider the time it takes for the population to evolve a proportion ( $k$ ) of the distance to equilibrium. When this distance is reached ( $t^*$ ), we have

$$\frac{\tilde{D}(t^*)}{\tilde{D}(0)} = 1 - k, \quad (12)$$

and this can be solved for  $t^*$  to obtain

$$t^* = \frac{\ln(1 - k)}{\ln\left[(1 - \hat{m})\left(1 - \frac{G}{\omega^2 + P}\right)\right]}. \quad (13)$$

We used equation (13) to consider the effect of  $G$  and  $\hat{m}$  on the time needed to evolve 90% of the distance ( $k = 0.9$ ) from the initial difference to the equilibrium difference. As  $G$  and  $\hat{m}$  increase, the time needed to reach equilibrium decreases (Fig. 3). Once  $G$  and  $\hat{m}$  reach moderate levels ( $h^2 = 0.3$ ,  $m = 0.05$ ), they have little further influence. When  $\hat{m} > 0.05$  or  $h^2 > 0.2$ , it generally takes fewer than 50 generations to evolve 90% of the distance to equilibrium (fewer than 10 generations to evolve 50% of the distance).

#### Assumptions

The quantitative-genetic theory of evolution and our extension of it assume a joint multivariate normal distribution of phenotypes and breeding values (Lande and Arnold 1983). This assumption may be valid within discrete populations, but is rarely tested. When breeding values from two different populations are mixed (as in the above), multivariate normality may be violated to a degree that depends on the difference in trait values between populations and on the degree

of mixing. A second assumption is constancy of  $G$  (and  $\mathbf{G}$ ). Selection on heritable characters should change genetic variance/covariance (Roff 1997, 2000). Moreover, mixing populations with different combinations of trait values will change  $\mathbf{G}$  to a degree that depends on the amount of mixing and the difference between populations (the difference in  $\mathbf{G}$  is likely small for closely related groups; Roff 2000). Temporal changes in  $\mathbf{G}$  are not relevant when considering equilibrium, but will influence the rate of approach to equilibrium. These two assumptions are unlikely to be seriously violated in empirical tests when either  $\hat{m}$  or  $D_0$  is small, which seems likely in many situations of empirical interest. When these conditions are not met, it may be useful to test for multivariate normality in phenotypes and, if possible, breeding values.

#### Comparisons to a Previous Model

King and Lawson (1995) developed a quantitative-genetic model of adaptive divergence for an island/continent scenario. Their model was similar to ours in its intended use for developing predictions of divergence in quantitative traits. Here we outline the King and Lawson (1995) model along with the equivalent equations from our model, and then discuss why ours is the correct formulation. We outline their model with corrections to their original equations, kindly provided by R. King. The originally published equations are provided in parentheses.

King and Lawson's (1995) model predicted that mean trait values on an island receiving immigrants from a continent should change as  $\Delta\bar{z} = G[\beta(1 - m) - dm]$  (not  $\Delta\bar{z} = G[\beta(1 - m) + dm]$ ), where  $G$  is the additive genetic variance,  $\beta$  is the selection gradient on the island,  $d$  is the difference in mean trait value between the island and the continent, and  $m$  is the proportion of the island population composed of immigrants. In the case of correlated characters,  $G$ ,  $\beta$ , and  $d$  are replaced by their matrix or vector equivalents. The equilibrium difference was found by setting  $\Delta\bar{z} = 0$  and solving for  $d$ , obtaining  $d^* = [(1/m) - 1]\beta$  (not  $d^* = [(1/m) - m]\beta$ ). This solution led King and Lawson (1995) to conclude that additive genetic variance does not influence the equilibrium difference. Their posited scenario parallels our SM model because they envision adults moving (i.e., selection before mixing). Assuming the continent population is at its optimum ( $\beta_j = 0$ ) and movement from the island to the continent is negligible ( $m_j = 0$ ), our equation (2) reduces to  $\Delta\bar{z} = md + (1 - m)G\beta$ , with an equilibrium solution of  $d^* = [(1/m) - 1]G\beta$  (adopting the notation of King and Lawson 1995). The critical distinction in our equation is that the equilibrium difference does depend on  $G$  (or  $\mathbf{G}$ ).

The fundamental point of departure between our model and that of King and Lawson (1995) centers on our use of breeding values. Breeding values are the additive genetic component of an individual's phenotype and can be thought of as the phenotypic value of an individual expressed as the average phenotypic value of its offspring (Falconer 1989; Roff 1997). When selection acts on a population, a nonrandom sample of adults survives, but because selection acts on phenotypes, the mean phenotype of individuals surviving an episode of selection will not equal the mean phenotype of their offspring. To determine the postselection contribution

of a group of parents to a mixture or a subsequent generation, the mean phenotype of the parents must be expressed as their mean breeding value. In this manner,  $G$  (or  $h^2$ ) should not be thought of as reproduction (as in King and Lawson 1995), but rather as the conversion of phenotypes to breeding values, which is necessary whenever selection takes place. Other quantitative-genetic models that consider the effects of mixing (albeit for different purposes) also convert phenotypes to breeding values, and have  $G$  (or  $h^2$ ) in the equilibrium solution (e.g., eq. A7 of Via and Lande 1985; eq. 6 of García-Ramos and Kirkpatrick 1997).

#### EMPIRICAL EXAMPLES

Traditional empirical approaches are often insufficient to infer a constraining role for population mixing in the adaptive divergence of quantitative traits. For example, the observation that differences between populations are not as large as would be expected based on differences between their respective environments does not assure that population mixing caused the discrepancy. Coupling an observation of apparent maladaptation with the measurement of effective number of migrants ( $N_e m$ ) remains inadequate because divergence in quantitative characters is directly influenced by  $m$ , not  $N_e m$ . The theoretical framework outlined above suggests more promising approaches. First, predicted equilibrium differences in trait values (e.g., using eqs. 3 or 5) can be compared to observed differences. Second, expected correlations between adaptive divergence and population mixing (e.g., Fig. 1A) can be compared to correlations observed among multiple, independent population pairs. Third, an experimental manipulation of population mixing (e.g., Riechert 1993) can be coupled with comparisons of predicted and observed shifts in mean phenotype. Here, we use data from two well-studied natural systems to illustrate the first two of these approaches. In each case, population mixing is estimated using presumed-neutral genetic markers to first estimate  $N_e m$  and from this  $m$ . To acknowledge that this approach provides an indirect estimate of population mixing, we use  $m$  rather than  $\hat{m}$  in the following.

##### *Lake Erie Water Snakes*

Water snakes live on mainland shores of Lake Erie and on off-shore islands. Snakes on the mainland inhabit heavily vegetated marshland and have strong contrasting color patterns (dark and light patches), whereas as those on islands inhabit exposed rocky shorelines and have reduced color patterns (patches reduced or absent; Camin and Ehrlich 1958). Selection imposed by avian predators has presumably led to the evolution of these differences because they increase crypsis, at least during some developmental stages (King 1992). However, some banded morphs are found on the islands, and snakes have been observed “swimming several miles from the nearest shore” (Camin and Ehrlich 1958, p. 507), suggesting immigration from the mainland could conceivably constrain adaptive divergence on the islands. To test this hypothesis, King and Lawson (1995) developed the quantitative-genetic model discussed above (as well as a model for Mendelian traits) and used it to predict the adaptive divergence of four color traits. Correspondence between predicted

TABLE 1. Observed trait differences between mainland and island water snakes in Lake Erie (King and Lawson 1995) and predictions from quantitative-genetic models of selection and population mixing ( $m$ , measured using allozymes). Traits include number of dorsal blotches (DB), number of lateral blotches (LB), height of lateral blotches in scale rows (ROWS), and extent of ventral pigmentation (VEXT). Values are in standard deviation units and represent mainland scores minus island scores. Predicted 1 scores were obtained using the quantitative-genetic model of King and Lawson (1995). Predicted 2 scores were obtained using our model, and assuming  $N = N_e$  ( $m = 0.01$ ). Predicted 3 and Predicted 4 scores were obtained using our model and different adjustments to  $m$  ( $N_e = 0.5N$ ,  $m = 0.02$ ;  $N_e = 0.1N$ ,  $m = 0.10$ ). Our model qualitatively predicts the correct direction of difference between populations, and under certain  $N_e/N$  assumptions, quantitative predictions are close to observed values.

Trait	Observed (SD)	Predicted 1	Predicted 2	Predicted 3	Predicted 4
DB	-0.8	-30.0	-8.1	-4.0	-0.7
LB	-0.9	-27.0	-11.0	-5.4	-1.0
ROWS	-2.8	1.0	-4.8	-2.4	-0.4
VEXT	-5.2	16.0	-2.1	-1.0	-0.2

and observed differences was fairly good for their Mendelian model (King and Lawson 1995), but was poor for their quantitative-genetic model (Table 1).

Here we use our quantitative-genetic framework with the empirical data collected by King and colleagues to develop new predictions of adaptive divergence for the four traits. The relevant solution from our model is  $\mathbf{d}^* = [(1/m) - 1]\mathbf{G}\boldsymbol{\beta}$  (see above), where  $\mathbf{d}^*$  is the vector of predicted differences between the island and mainland snakes,  $\mathbf{G}$  is the additive genetic variance/covariance matrix for the traits (provided in King and Lawson 1995; from King 1993a),  $\boldsymbol{\beta}$  is the vector of selection gradients in the island population (provided in King and Lawson 1995; from King 1993b), and  $m$  is the proportion of the island population composed of immigrants. King and Lawson (1995) estimated  $m$  using data on allozyme variation to calculate  $N_e m$ , which was then divided by the estimated adult population size ( $N$ ). In natural populations,  $N_e$  is usually less than  $N$ , suggesting that it might be more appropriate to divide  $N_e m$  by  $N_e$ . Unfortunately, estimates of  $N_e$  are not available for the water snake populations. Instead, we estimated  $N_e$  from  $N$  using two  $N_e/N$  ratios that bracket the possibilities (0.1, Frankam 1995; 0.5, Nunney 1995). These ratios yielded estimates of  $m = 0.1$  and  $m = 0.02$ , respectively, in contrast to the value of  $m = 0.01$  that King and Lawson (1995) obtained using  $N$ .

The predictions obtained using our model were much closer to the observed values than were the predictions obtained using King and Lawson’s (1995) model. First, our model correctly predicted the direction of difference for all four traits (Table 1). This improvement over King and Lawson (1995) was the result of  $\mathbf{G}$  appearing in our equilibrium solution. Thus, traits under direct selection to be higher on the islands can nevertheless be lower at equilibrium because of their positive genetic covariance with traits under strong selection to be lower on the island. Second, using  $N_e$  when estimating  $m$  further improved the fit for several traits. If  $m$  was decreased in the model, correspondance between predicted and equilibrium values worsened, suggesting that population mixing is constraining adaptive divergence (also con-

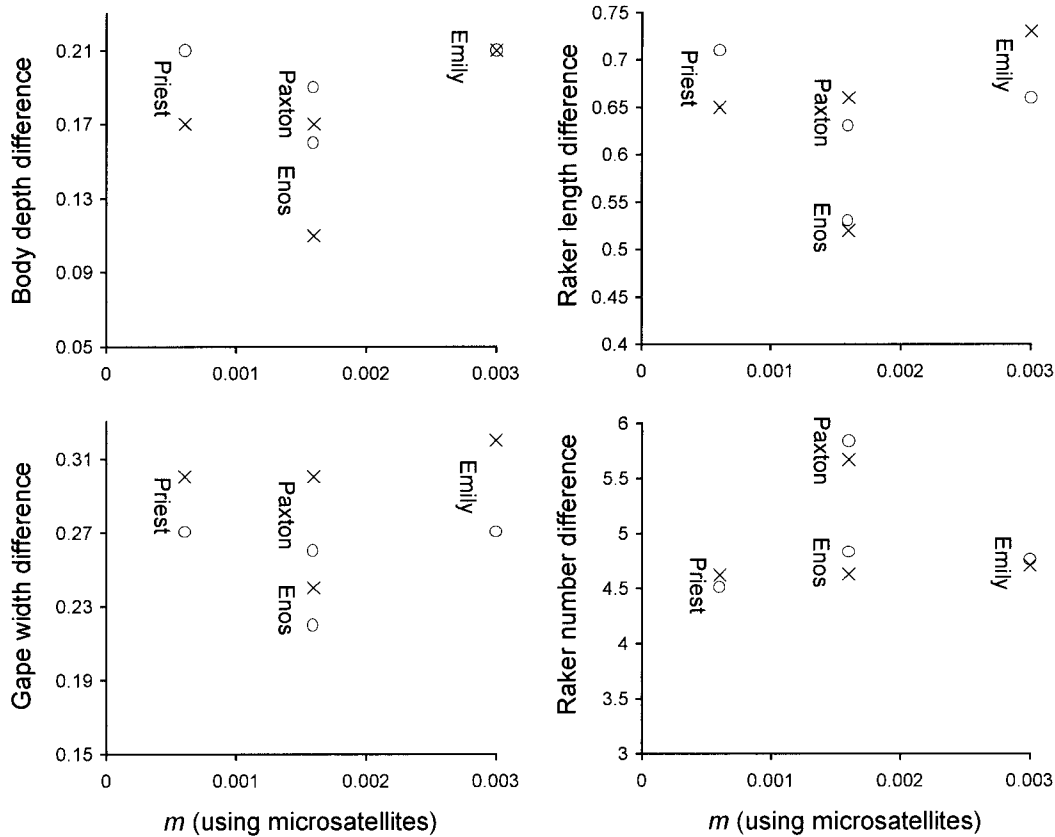


FIG. 4. Adaptive divergence versus population mixing (estimated using microsatellites) between benthic and limnetic stickleback in each of four lakes (Enos, Emily, Paxton, Priest). Each  $m$  trait difference is expressed as an absolute value for males (open circles) and females (crosses). No association between divergence and gene flow is evident.

cluded by King and Lawson 1995). Analysis of the water snake data shows that empirical estimates of selection,  $G$ , and population mixing can provide accurate qualitative predictions (i.e., the direction of divergence) and perhaps even reasonable quantitative predictions if the relevant parameters are measured with enough accuracy.

*Benthic and Limnetic Stickleback*

Two forms of threespine stickleback occur sympatrically in several small lakes along the southern coast of British

Columbia (Schluter and McPhail 1992), and each pair has evolved independently (Taylor and McPhail 1999, 2000). One form (limnetic) is specialized for feeding on zooplankton in the open water, and the other form (benthic) is specialized for feeding on benthic macroinvertebrates along lake margins. Relative to the limnetic form, the benthic form has a more robust body, a larger mouth, and fewer, shorter gill-rakers—differences known to facilitate their different feeding modes (Schluter and McPhail 1992). We used these benthic/limnetic pairs to illustrate the correlation approach when testing for an effect of population mixing on adaptive divergence.

We estimated adaptive divergence between the forms within each lake using mean values for traits associated with swimming ability (body depth) and trophic morphology (gape width, gill-raker length, gill-raker number; from the Appendix of Schluter and McPhail 1992). The various benthic/limnetic pairs varied considerably in the extent of their morphological divergence, with similar patterns for males and females (Fig. 4). The relative amount of trait divergence between the pairs was roughly similar for body depth, gape width, and gill-raker length (Emily > Priest > Paxton > Enos), but differed for gill-raker number (Fig. 4).

To estimate population mixing for each benthic/limnetic pair, we first obtained  $F_{ST}$ -values from Taylor and McPhail (2000) and converted these to  $N_e m$  using Wright's (1978) standard formula (Table 2). This approach has many assumptions (see Whitlock and McCauley 1999) that benthic/

TABLE 2. Estimates of genetic differentiation and parameters used to estimate gene flow between benthic and limnetic sticklebacks within each of four lakes.  $F_{ST}$ -values were estimated using six microsatellite loci (Taylor and McPhail 2000).  $N_e m$  was estimated from these values as  $[(1/F_{ST}) - 1]/4$ . The total population size of sticklebacks in each lake was estimated using lake surface area and Reimchen's (1990) estimate of 670 stickleback per hectare (divided by two to obtain an average for each type). Effective population size ( $N_e$ ) was estimated as  $0.1N$  and mixing rate ( $m$ ) as  $N_e m/N_e$ .

Lake	$F_{ST}$	$N_e m$	Lake area (ha)	$N$	$N_e$	$m$
Enos	0.2086	0.9285	17.6	5893	589	0.0016
Paxton	0.2127	0.9254	17	5692	569	0.0016
Priest	0.2086	0.9485	44	14,732	1473	0.0006
Emily	0.3356	0.4949	5	1692	169	0.0030

limnetic pairs probably violate, but as long as the assumptions are violated in a similar way for each pair, relative  $N_e m$  values are likely comparable. In general,  $N_e m$  was quite low between the two forms in each lake ( $0.50 < N_e m < 0.95$ ; Table 2), suggesting very limited genetic exchange.

Our next step was to convert the estimates of  $N_e m$  to  $m$ . Estimates of  $N_e$  are not available, so we used an approach similar to that adopted for water snakes. Reimchen (1990) used extensive mark-recaptures to estimate that 75,000 adult stickleback were present in a 112-ha bog lake on the Queen Charlotte Islands, British Columbia. Using this estimate of 670 stickleback per hectare, we estimated total  $N$  for each of the four benthic/limnetic lakes based on their surface area (Table 2). These estimates appear fairly accurate (at least within a factor of two) because the estimate for Enos Lake is comparable to mark-recapture estimates within that lake (B. Matthews and D. Schluter, unpubl. data). We assumed benthic and limnetic stickleback have roughly similar population sizes (B. Matthews and D. Schluter, unpubl. data) to then estimate average  $N$  per type. We converted  $N$  to  $N_e$  by assuming  $N_e = 0.1N$  (Frankham 1995; but see Waples 2001). The use of a different ratio would not have influenced our conclusions. We divided  $N_e m$  by  $N_e$  to obtain estimates of  $m$  for each pair, which were plotted against divergence for each trait (Fig. 4).

No clear association was evident between adaptive divergence and  $m$  (Fig. 4). The lack of a correlation could arise because: (1) the models are incorrect or the assumptions are violated; (2) the estimates of adaptive divergence are incorrect; (3) the estimates of  $\hat{m}$  are incorrect; or (4) population mixing is currently not important to divergence. Each of the first three possibilities could of course be true, but we favor the last because population mixing was so low ( $F_{ST} = 0.21$ – $0.34$ ,  $N_e m < 1.0$ ,  $m < 0.004$ ) that the pairs would all be very near their optima. If the optima differed among pairs, the resulting variation in equilibrium differences will bear little relation to population mixing. We conclude that benthic/limnetic stickleback are a case where population mixing does not currently have an appreciable influence on adaptive divergence.

#### FUTURE WORK

We illustrated several ways in which theory can be used to aid empirical tests for whether population mixing between spatially-discrete populations constrains the adaptive divergence of quantitative traits. We hope this demonstration encourages other investigators to make further improvements. A remaining ambiguity is the precise relationship between the movement of individuals (population mixing), the movement of genes (gene flow), the parameter  $\hat{m}$ , and the estimator  $m$ . Another important question is how to distinguish between different scenarios that can cause a negative correlation between gene flow ( $m$ ) and adaptive divergence. In one scenario, the strength of divergent selection may be stronger between some population pairs, leading to the evolution of increased reproductive isolation and lower gene flow between them, even if population mixing itself remains constant. This is the principle of ecological speciation, which has been tested using the correlation approach (Orr and Smith 1998; Lu and

Bernatchez 1999). In another scenario, intrinsic variation in population mixing (for example, different geographic distances between pairs) determines the extent of gene flow and therefore adaptive divergence. This is the scenario considered in the present paper. Finally, effects of population mixing (and gene flow) on the distribution of breeding values and the structure of the  $\mathbf{G}$  matrix should be examined, along with implications for the application of quantitative-genetic models. Tests for the effects of population mixing on adaptive divergence are critical to many issues in ecology, evolution, and conservation. We hope that our illustration of a theoretical framework to aid such tests will provide a better fusion between theory and empiricism in future endeavors.

#### ACKNOWLEDGEMENTS

We thank S. Latham, A. Poon, D. Schluter, and M. Whitlock for formative discussions regarding the quantitative-genetic models. B. Matthews and D. Schluter shared preliminary estimates of population sizes for benthic and limnetic sticklebacks. Useful comments on the manuscript were provided by L. Bernatchez, N. Johnson, T. Lenormand, and two anonymous reviewers. We also thank R. King, whose original model (King and Lawson 1995) helped inspire our models; he commented at length on a draft of this manuscript and provided us with an unpublished manuscript of his own (written with A. Queral-Regil). Despite our disagreement regarding model formulation, King's ideas were important in the development of our own. APH was supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC). TD and ET received research support from NSERC operating grants.

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Corresponding Editor: C. López-Fanjul