#### ONLINE APPENDIX A

#### *Testing parallel evolution*

Here we describe how we calculated the variance components for the habitat main effect and the habitat  $\times$  lineage interaction in the fitted two factor ANOVA (see equation [1] in the main text), and how we used these values to estimate  $\gamma$ .

The  $\alpha_i$  in equation [1] are the habitat main effects of the two factor ANOVA model, with corresponding variance component

$$A = \frac{b}{a - 1} \sum \alpha_i^2 \ . \tag{A1}$$

The constants a and b are the total numbers of habitat types and lineages, respectively, in the data set. For our data, a = 2 and b = 2. The corresponding variance component for the interaction of the two factors, lineage and habitat type, is

$$AB = \frac{1}{(a-1)(b-1)} \sum_{i} (\alpha \beta)_{ij}^{2} . \tag{A2}$$

The above variance components A and AB were estimated from the fitted data. The procedure is simplest in the case of a balanced design (equal sample size for all habitat and lineage combinations): calculate the fitted estimates by applying the formulas for the expected mean squares (Sokal and Rohlf 1995, p333) to the observed mean squares provided in the ANOVA table and solve for A and AB. In the more typical case of unequal sample sizes, as was the case here, it is easiest to calculate the variance components directly from the estimated ANOVA model parameters,  $\alpha_i$  and  $(\alpha\beta)_{ij}$ . We extracted these parameter estimates from the fitted model using the *dummy.coef* command in S-Plus 6.0 (Insightful Corporation 2002). Plugging these estimates into equations [A1] and [A2] yielded estimates of the habitat and interaction variance components, A and AB.

These estimates of A and AB were plugged into equation [2] in the main text to yield the estimate of variance proportion  $\gamma$ , here employed as a coefficient of parallel evolution. Figure A1 shows the relationship between changes in mean trait values of two lineages across a single environmental transition, the relative magnitudes of the associated variance components, and the corresponding proportion  $\gamma$ .

Confidence limits for the estimate  $\hat{\gamma}$  were calculated using the bootstrap (Efron and Tibshirani 1986). On each iteration of this procedure a new sample of the data was generated by resampling with replacement from the original data, while retaining the same sample size for each combination of habitat and lineage. Resampling was repeated 1000 times, leading to a new value of  $\hat{\gamma}$  each time. The standard deviation of the 1000 values is the standard error of  $\hat{\gamma}$ .

### Testing parallel inheritance

Here we summarize the methods for assessing goodness of fit of line means and line mean absolute deviations (MADs) to the regression models used to test parallel inheritance (equations [3] and [4] in main text).

Analysis of line means.— The regression equation [3] fitted to line means is written in matrix form as

$$\overline{\mathbf{z}} = \mathbf{M}\mathbf{a} + \mathbf{e} . \tag{A4}$$

Here,  $\overline{\mathbf{z}}$  is the vector of line means.  $\mathbf{M}$  is the matrix of indicators  $M_{ij}$ , which represent the contributions of the constant  $(\mu)$ , additive  $(\alpha)$ , and dominance  $(\delta)$  components of variation

among line means. The vector **a** contains the three model parameters to be estimated:  $\mu$ ,  $\alpha$ , and  $\delta$ . The vector **e** contains the deviations  $e_i$ .

Goodness of fit of regression models to line means was tested in the following way. First, the coefficients in **a** were estimated using generalized least squares (Lynch and Walsh 1997),

$$\hat{\mathbf{a}} = (\mathbf{M}^T \mathbf{V}^{-1} \mathbf{M})^{-1} \mathbf{M}^T \mathbf{V}^{-1} \overline{\mathbf{z}}. \tag{A5}$$

V is a diagonal matrix containing the squared standard errors of observed line means in  $\bar{z}$ . Superscripts T and -1 indicate transpose and inverse, respectively. Second, the estimate  $\hat{a}$  was used to calculated the predicted means,

$$\hat{\mathbf{z}} = \mathbf{M}\hat{\mathbf{a}} . \tag{A6}$$

Finally, we calculated the goodness of fit statistic,

$$\chi^2 = \sum_{i=1}^n \frac{(\overline{z}_i - \hat{z}_i)^2}{\operatorname{var}(\overline{z}_i)},\tag{A7}$$

where  $\bar{z}_i$  is the observed trait mean in the  $i^{th}$  line (contained in the vector  $\bar{z}$ ),  $\hat{z}_i$  is the corresponding predicted line mean (contained in  $\hat{z}$ ), and  $\text{var}(\bar{z}_i)$  is the squared standard error of the observed trait mean. The test statistic  $\chi^2$  has a  $\chi^2$  distribution with degrees of freedom equal to the number of lines (8) minus the number of estimated parameters (2 parameters in the constant plus regional differences model; 3 parameters when the additive component is included; and 4 parameters when the dominance component is also included). A small and non-significant  $\chi^2$  value indicated a good fit. Conversely, a large and significant  $\chi^2$  value was regarded as indicating a poor fit.

Analysis of line variances.— The regression equation [4] fitted to line mean absolute deviations (MAD) is written in matrix form as

$$\overline{\mathbf{w}} = \mathbf{M}\mathbf{a} + \mathbf{e} . \tag{A8}$$

 $\overline{\mathbf{w}}$  is the vector of line MADs.  $\mathbf{M}$  is a matrix of indicators  $M_{ij}$ , which represent the contributions of the constant  $(\mu)$ , hybrid expansion  $(\kappa)$ , and segregation variance  $(\varphi)$  components of variation among line MADs. The vector  $\mathbf{a}$  contains the three model parameters to be estimated:  $\mu$ ,  $\kappa$ , and  $\varphi$ . The vector  $\mathbf{e}$  contains the random errors. Goodness of fit of this model to data was evaluated using the same procedures as in the analysis of line means, explained above.

## Estimating minimum effective number of loci

After pooling the data from the two regions on lateral plates in crosses between adjacent marine and stream populations, we estimated the minimum effective number of loci from the observed segregation variance in the  $F_2$  lines. Here we briefly explain the methods we used.

We followed the approach used by Hatfield (1997), based on Lande (1981) and others.

We estimated gene number using the formula

$$\hat{n}_e = \frac{(\overline{z}_M - \overline{z}_S)^2 - \text{var}(\overline{z}_M) - \text{var}(\overline{z}_S)}{8[\text{var}(F_2) - \text{var}(F_1)]},\tag{A9}$$

where  $\bar{z}_M$  and  $\text{var}(\bar{z}_M)$  are the mean and squared standard error of the mean of the pooled marine parents,  $\bar{z}_S$  and  $\text{var}(\bar{z}_S)$  are the mean and squared standard error of the mean of the pooled stream parents, and  $\text{var}(F_1)$  and  $\text{var}(F_2)$  are the pooled variances of the  $F_1$  and  $F_2$  offspring. Confidence limits for the estimate  $\hat{n}_e$  were calculated using the bootstrap (Efron and Tibshirani 1986). On each iteration of this procedure a new sample of the data was generated by resampling with replacement from the original data, while retaining the same sample size for each cross. Resampling was repeated 1000 times leading to a new value for  $\hat{n}_e$  each time. The

standard deviation of the 1000 values is the standard error of  $\hat{n}_e$ . The 2.5 percent and 97.5 percent quantiles of the 1000 values is an approximate 95 percent confidence interval for  $\hat{n}_e$ .

# References

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Figure A1. Possible outcomes involving two independent, closely related lineages diverging between two environments. The quantity  $\gamma$  is used as an index of parallel evolution, and is the difference between the variance component for habitat in the fitted model (A) and the variance component for the interaction between habitat and lineage (AB), expressed as a proportion of the sum of these two variance components (cf. equation [2] in main text). Parallel evolution has occurred if  $\gamma > 0$ , that is, if the variance component for habitat exceeds that for the interaction of habitat and lineage. The three scenarios shown represent the extremes of: a) pure parallel evolution, where lineages respond identically to an environmental transition (A > 0, AB = 0); b) no parallel evolution (A = AB = 0); and c) no parallel evolution, with lineages responding in opposite ways to the environmental transition (A = 0, AB > 0; "anti-parallel" evolution).

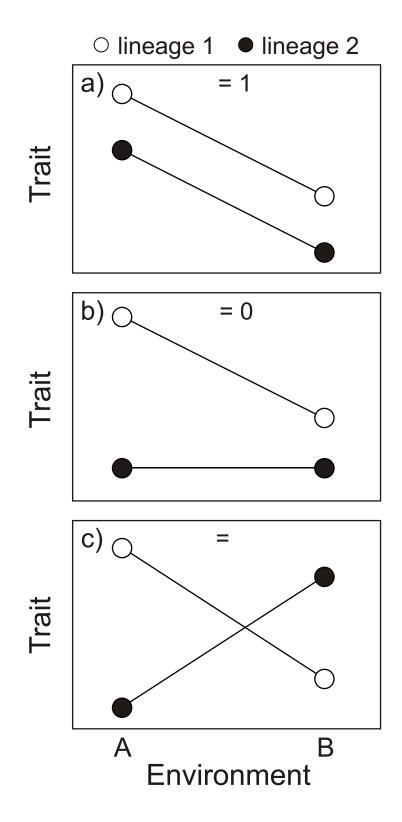


Fig A1 Schluter et al