GENETIC DIVERGENCE IN ADAPTIVE CHARACTERS BETWEEN SYMPATRIC SPECIES OF STICKLEBACK

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Submitted June 17, 1996; Revised October 15, 1996; Accepted October 29, 1996

Abstract.—This study explored the genetic basis of phenotypic differences between two sympatric species of ecologically and morphologically divergent sticklebacks (*Gasterosteus aculeatus* complex). The aim was to understand how many loci determine the differences and to what extent the differences are due to additive or nonadditive gene action. I reared the two parental species, F_1 and F_2 hybrids, and both backcrosses in the laboratory and measured the following quantitative characters: gill raker number and length (both involved in feeding), lateral plate number and pelvic spine length (both involved in predator defense), and growth (a fitness component). I then applied joint-scaling regression models to estimate composite additive, dominance and epistatic effects, and their contribution to divergence of parental lines. A simple additive model was sufficient for gill raker number and growth; additive and dominance effects contributed significantly to divergence in plate number and pelvic spine length; and additive, dominance, and epistatic effects contributed significantly to divergence in gill raker length. Wright's estimator for the number of loci for the four morphological characters ranged from 1 to 50. My results suggest that adaptive divergence between limnetic and benthic sticklebacks has taken place through a variety of genetic mechanisms specific to different traits. Though interspecific hybrids are completely fertile and viable in the laboratory, they are selected against in the wild. The pattern of inheritance for the traits examined here directly influences how well hybrids can exploit the two major resource environments in the wild.

A basic goal in evolutionary biology is to understand the genetic basis of differences among species: what are the differences and how did they arise? Descriptions of divergence are of interest on their own, but knowledge of the number of genes that underlie species differences and the strength of interactions among loci would be helpful in several ways. First, knowledge of genetic differences among species allows us to predict the tempo of evolutionary change. Genetic divergence arises because of natural selection, genetic drift, mutation, and/or gene flow, and knowledge of genetic divergence allows us to predict evolution in the face of these forces. Second, knowledge of species differences informs modeling of quantitative character evolution. Most models of the evolution of quantitative characters assume a particular genetic architecture: that traits are polygenic and variation is entirely additive (e.g., Lande 1980*a*, 1980*b*, 1982; Cheverud 1984; Rose 1985; Via and Lande 1985; Gillespie and Turelli 1989;

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Am. Nat. 1997. Vol. 149, pp. 1009–1029.

Charlesworth 1990). We currently have little idea of how often this is true and, when it is not, how severe the violations or the consequences of these violations are. Third, knowledge of genetic differences among species is essential if we are to disentangle genetic and ecological causes of reproductive isolation. For example, hybrids may be selected against ecologically because of an intermediate phenotype (e.g., Grant and Grant 1993; Schluter 1993, 1995; Hatfield 1995). Understanding the inheritance of hybrid phenotypes is a step toward understanding the genetic basis of ecological causes of reproductive isolation.

Evidence seems to be building against the assumption that traits are polygenic and variation is entirely additive. Orr and Coyne (1992) reviewed the evidence for polygenic inheritance of adaptive traits and found that there is, in fact, little empirical support for the neo-Darwinian view that such traits are coded for by many genes of small effect. Their basic conclusion was that "while some adaptations are clearly based on many genes of small effect, major genes are also sometimes involved" (p. 736). A similar position is building against the idea that additive genetic variation is the only component underlying adaptive quantitative traits. For example, high heritabilities (a measure of additive genetic variation) may exist despite many epistatic gene interactions (Long et al. 1995): there is a difference between the additive genetic variance for a trait and the nature of the effects underlying that trait. It remains unclear whether the presence of epistatic interactions should alter our view of evolutionary processes: if genes act in a predominantly additive fashion, then discovery of weak genetic interactions may not be sufficient to alter our views.

The primary objective of the study I present here is to describe the genetic basis of differences between two closely related species of three-spined sticklebacks. These species have diverged in a number of characters, probably mainly through resource competition and adaptation to different resource environments (McPhail 1984, 1992, 1993, 1994; Schluter and McPhail 1992; Schluter 1993, 1994, 1995; Hatfield 1995) and the predators encountered there. By crossing the species and measuring mean and variance of quantitative traits, I explored how the two species have diverged at loci determining two feeding characters (gill raker number and gill raker length), two armor characters (plate number and pelvic spine length), and one fitness component (growth). I used regression techniques to examine the contribution of additive, dominance and epistatic effects to the differences in species means. I also used Wright's (1968) biometric technique to estimate the effective number of loci responsible for the differences among species means in the four morphological traits, and I used a bootstrap technique to provide lower bounds for these estimates.

THE STUDY SPECIES

Three-spined sticklebacks (*Gasterosteus aculeatus* complex) are small fish common to coastal marine waters throughout the Northern Hemisphere (Bell and Foster 1994). Marine sticklebacks gave rise to populations in many coastal lakes and streams at the end of the Pleistocene (Bell and Foster 1994; McPhail 1994). Several lakes on islands in the Strait of Georgia, British Columbia, Canada, are exceptional because they contain sympatric species pairs of stickle-

backs, whereas most lakes contain only solitary populations (McPhail 1984, 1992, 1993, 1994; Schluter and McPhail 1992). Each species pair exhibits a similar pattern of morphological divergence (McPhail 1984, 1992, 1993, 1994; Schluter and McPhail 1992). Evidence suggests that divergence has been driven by natural selection. In specific terms, the species are character displaced in each lake (Schluter and McPhail 1992), there is experimental evidence of sharp trade-offs in resource use efficiency and selection due to competition for resources (Schluter 1993, 1994, 1995), and postzygotic isolation is due to ecologically driven selection: interspecific hybrids have high fitness in the laboratory but are selected against in the wild because of their lower foraging success in the two main habitats (McPhail 1992; Schluter 1993, 1995; Hatfield 1995).

We refer to these species by their preferred foraging habitats. Limnetics exploit primarily plankton, and benthics exploit mainly benthic prey in the littoral zone. Limnetics have a fusiform body, narrow gape, and many, long gill rakers; benthics have a robust body form, wide gape, and few, short gill rakers (McPhail 1984, 1992, 1994; Schluter and McPhail 1992). Gill rakers are the feeding apparatus of fish, and gill raker number and length are heritable in sticklebacks (Hagen 1973; D. Schluter, unpublished data). Long, numerous gill rakers are prevalent in planktivorous fish in general (Magnuson and Heitz 1971; Kliewer 1970; Sanderson et al. 1991; Schluter and McPhail 1993).

Limnetics and benthics have also diverged in armor characteristics (McPhail 1984, 1992, 1994). Benthics exhibit armor reduction sometimes to the point of a complete lack of lateral plates and pelvic and dorsal spines, whereas limnetics have long spines and high plate counts. Divergent armor characteristics are assumed to reflect divergent predation pressures between limnetics and benthics since armor variation is heritable in sticklebacks (Hagen 1973). Insect predators tend to hunt in the covered littoral habitat exploited by benthics, and individuals with little or no armor offer few sites for these predators to grab hold (Reist 1980; Zyuganov 1989). On the other hand, limnetics forage in the open and so are exposed to predation by piscivorous trout and birds. They are therefore expected to have more extensive armor (see Hagen and Gilbertson 1972; Bell and Haglund 1978; Reimchen 1980, 1988, 1992, 1994).

Geological and molecular evidence suggests that each pair has been independently derived (McPhail 1993; Schluter and McPhail 1993; Taylor et al., in press). Evidence that these are good species under the biological species concept is discussed by McPhail (1994). For example, the species maintain their differences over at least two generations in a common environment (McPhail 1984, 1992; Hatfield 1995) despite some phenotypic plasticity (Day et al. 1994). In this article, I present results from crosses between the two species from Paxton Lake on Texada Island, British Columbia.

MATERIAL AND METHODS

Experimental Crosses

All fish used in these analyses were reared entirely in the laboratory under conditions conducive to excellent growth and survival and containing elements

of both the benthic and limnetic wild environments (Hatfield 1995). The fish were reared in 102-L aquaria divided in two with fine mesh screening to allow the rearing of two separate families per tank. Each side was supplied with its own power filter and airstone. Identical conditions were used for all groups to minimize variability caused by phenotypic plasticity (Day et al. 1994).

I made full-sib crosses by combining eggs and macerated testes in a petri dish with a small volume of water. I then randomly selected 30 eggs from the fertilized clutch and placed them in a mesh-bottomed 250-mL container attached to the side of a randomly assigned aquarium, aerating them from below using an airstone. As fish hatched, they were removed from the hatching container and placed in the tank. Hatchlings were fed for the first few days on infusoria cultures then transferred to diets of live *Artemia* nauplii and frozen crustaceans and insect larvae. Fish were fed twice daily to satiation to avoid competition for food. Under these laboratory conditions, survival is very high for hybrid and parental crosses (Hatfield 1995).

After 6 mo of growth, fish were simultaneously brought into reproductive condition by manipulation of temperature and light (Baggerman 1957; Hatfield 1995). This involved taking the fish through an approximate 3-mo period of low temperature and short day length (4°–10°C; 8L:16D), followed by a gradual return to the original temperature and light regime (18°C; 16L:8D) typical of summer conditions for these species in the wild.

I made the following crosses: pure limnetic (16 families), pure benthic (15 families), F_1 (limnetic × benthic; 21 families) and F_2 ($F_1 \times F_1$; 31 families) hybrid crosses, and limnetic and benthic backcrosses (limnetic × F_1 and benthic × F_1, respectively; eight families of each). These six cross types, or "lines," are referred to as P_{lim}, P_{ben}, F_1, F_2, B_{lim}, and B_{ben}, respectively. The F_1 hybrid lines included both reciprocal crosses, and the F_2 hybrid lines included all four reciprocal crosses, but because of rearing space restrictions, I backcrossed only male F_1 hybrids with the parental species (i.e., two of four possible reciprocal crosses; Hatfield 1995). No sibs were ever crossed, and families in the F_2 and backcross lines were made with parents from randomly chosen (without replacement) families in the P_{lim}, P_{ben}, and F_1 lines.

The first generation (i.e., P_{lim}, P_{ben}, and F_1) was made in April–May 1992 using gametes from wild caught fish from Paxton Lake. The wild-caught fish were discarded and not used in any of the analyses. The first generation was raised to maturity (1 yr) and then was used to make the second generation (F_2, B_{lim}, and B_{ben} plus some additional P_{lim}, P_{ben}, and F_1 families) in April–May 1993 before being preserved. Second-generation fish were raised for approximately 7 mo and then preserved. Because they were not reproductively mature, they could not be sexed.

Fish were anesthetized in MS222, fixed in ≈4% formaldehyde for at least 1 wk, stained with alizarin red, and finally stored in 40% isopropyl alcohol. I measured morphology using a dissecting microscope fitted with an ocular micrometer. Gill raker number is the total on the first gill arch; gill raker length is length of the longest raker on the first gill arch; plate number includes all staining plates from left and right sides regardless of size; pelvic spine length is total

length from tip to hinge. Gill rakers and pelvic spines were measured on the left side. Repeatability is extremely high for these characters (Hatfield, in press).

Only gill raker length and pelvic spine length varied significantly with body size over the range of fish sizes examined here (T. Hatfield, unpublished data), so I size-corrected them using a method modified from Schluter and McPhail (1992). I first ln-transformed gill raker length and pelvic spine length along with three measurements of body size: standard body length, body depth, and mouth width. A general body size variable was calculated as PC1 from a principal component analysis using ln-transformed body length, body depth, and mouth width. Size-corrected spine and gill raker length were then calculated as residuals from linear regression of each trait on PC1. Performing ln-transformation before size correction ensured that spine and gill raker length were linearly related to overall size and had normal distributions with equal variances among lines. Conducting ln-transformation for plate number and gill raker number was not necessary, but results are the same if transformed.

I measured growth, a fitness-related trait, in addition to trophic and armor characters. Growth was measured in 1993 only. The number of families per line for growth measurements are therefore somewhat smaller than for trophic and armor characters, as follows: limnetic ($n = 5$), benthic ($n = 6$), F_1 hybrid ($n = 9$), F_2 hybrid ($n = 32$), limnetic backcross ($n = 7$), and benthic backcross ($n = 7$). The number of fish in each family was nevertheless large ($\bar{X} = 22.5$ individuals). Eighteen weeks after fertilization, I weighed each family and calculated a per-individual mass (i.e., family weight/no. of individuals). Because all crosses hatch at approximately the same very small size (eggs are <2 mm in diameter and <350 μg; Wooton 1973), mass at 18 wk ($\bar{X} = 0.364$ g, SD = 0.080, water displacement mass) provides an estimate of total growth.

Density varied among tanks because of mortality during embryo development and shortly after hatch, but approximately the same range of densities occurred in each line. Growth had a strong nonlinear relationship with density. I corrected for density by using residuals from a locally weighted regression (lowess algorithm, S-plus; Statistical Sciences 1991) of growth on density. I used these residuals for all analyses of growth.

Analysis of Line Means

I maintained statistical independence of data in all analyses by using family means rather than combining all measured individuals. This is a conservative step. Sample sizes for all tests are the number of families in each line.

I tested for sex differences in benthics, limnetics, and F_1 hybrids using two-sample *t*-tests. I tested for maternal effects by comparing character means of males from the two reciprocal F_1 crosses using two-sample *t*-tests. Since second-generation fish were not sexed, I could not assess sex differences in growth, and sample size was inadequate to test for maternal effects in growth. Maternal effects tested in this way may include sex-linked effects.

The tests indicated that differences in sex were not significant ($\alpha = 0.05$) for any of the traits. I therefore pooled data from both sexes for subsequent analyses. The tests for maternal effects were not significant for gill raker number and

length or plate number, so I also pooled reciprocal crosses within each line. Maternal effects were detected for pelvic spine length ($P = .01$) and remained marginally significant at $\alpha = 0.05$ after Bonferroni correction (Rice 1989). I removed this effect before further analysis by subtracting the mean difference from one of the F_1 reciprocal crosses. I also tried several variants on this approach (e.g., assuming the maternal effect was additive and removing the expected effect from each of the lines). Conclusions based on these transformations are the same as those for the untransformed data (i.e., pooled reciprocal crosses) probably because for this character the maternal effect was very small: mean spine length of the reciprocal crosses differed by <7% of the difference in spine length between benthics and limnetics. I present only the results from analyses of pelvic spine length using pooled reciprocal crosses.

I tested for additivity, dominance, and epistasis and their contribution to divergence of parental lines using joint scaling, a regression technique developed by Cavalli (1952) and Hayman (1958, 1960*a*, 1960*b*). The analysis was carried out on differences between limnetics and benthics in all five traits. Additive effects alone cause the mean phenotype of every line to be the average of the means of its parental lines (an example will be shown later in fig. 1*A*). Dominance effects cause means of all hybrid lines to resemble one parent more than the other. This tendency is stronger in the F_1's than in the F_2's and backcrosses (an example will be shown later in fig. 1*C*). Epistasis causes hybrid line means to deviate significantly from expectations of additivity or dominance. All three effects may occur simultaneously.

The joint-scaling test for phenotype means is summarized in Mather and Jinks (1982) and Lynch and Walsh (1997). Although originally developed for genetic analysis of divergent inbred lines, the method can be used for the analysis of interfertile wild populations provided that close relatives are not mated (Hard et al. 1992). Briefly, the technique fits the following multiple regression model to the observed line means,

$$z_i = \mu + \mathbf{M}_{i2}\alpha + \mathbf{M}_{i3}\delta + \epsilon_i, \quad (1)$$

where z_i is the trait mean in the ith line, μ is the mean of all line means, α is the additive genetic effect, δ is the effect of dominance, and ϵ_i is the sampling error associated with the ith line. When epistasis terms (i.e., additive × additive, dominance × dominance, etc.) are absent from the model, they are implicitly included in the error term along with sampling error. (Note that in statistical genetics, effects are always defined in this hierarchical fashion, with additive effects coming first, etc.; Fisher 1918; Cockerham 1954; Kempthorne 1954.) Also, $\mathbf{M}$ is a matrix of coefficients specifying predicted line means under the additive and additive-plus-dominance models. The first column in $\mathbf{M}$ is all 1's. The second column ($\mathbf{M}_{i2}$ in eq. [1]) contains coefficients for additive effects, where the two parental lines are -1 and 1, respectively, the F_1's and F_2's are taken as 0 (i.e., under additivity these means are expected to be halfway between the parental means), and backcrosses are -0.5 and 0.5, respectively (i.e., backcross means are expected to be halfway between the parental and F_1 means). The third column ($\mathbf{M}_{i3}$ in eq. [1]) contains coefficients for dominance effects. The coeffi-

cients here are 1 for each of the parental lines, 1 for the F_1's, and 0 for the F_2's and backcrosses. Thus, dominance effects are expected to shift the F_1 mean two units and the F_2's and backcrosses half as much.

The regression model is fit using the standard expression for weighted (generalized) least squares,

$$\hat{\boldsymbol{a}} = (\mathbf{M}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{M})^{-1}\mathbf{M}^{\mathrm{T}}\mathbf{V}^{-1}\bar{\mathbf{z}} \tag{2}$$

and

$$\hat{\boldsymbol{z}} = \mathbf{M}\hat{\boldsymbol{a}}, \tag{3}$$

where $\hat{\boldsymbol{a}}$ is a vector containing estimates of the three model parameters, μ, α, and δ; $\mathbf{M}$ is the matrix of coefficients from equation [1]; $\mathbf{V}$ is a diagonal weighting matrix of sampling variances of observed line means (squared standard errors); $\bar{\boldsymbol{z}}$ is the vector of observed line means; and $\hat{\boldsymbol{z}}$ is the vector of predicted line means. (Data from the lines are therefore in $\bar{\boldsymbol{z}}$ and $\mathbf{V}$.) Superscripts T and -1 indicate transpose and inverse, respectively. Standard errors of parameter estimates are square roots of the diagonal elements of $(\mathbf{M}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{M})^{-1}$ (Lynch and Walsh 1997).

I tested the fit of regressions by comparing observed and predicted line means, using the following goodness-of-fit test statistic (Mather and Jinks 1982; Lynch and Walsh 1997):

$$\chi^2 = \sum_{i=1}^{n} \frac{(\bar{z}_i - \hat{z}_i)^2}{\mathrm{var}(\bar{z}_i)}, \tag{4}$$

where the degrees of freedom are the number of lines (six) minus the number of estimated parameters (i.e., df = 3 for the additive-plus-dominance model, df = 4 for the additive model, and df = 5 for the model of no effect [i.e., $z_i = \mu + \epsilon_i$]).

I tested genetic models by sequential model fitting (Lynch and Walsh 1997), beginning with the model of no effect. Rejection of this model by the above goodness-of-fit test indicates genetic divergence among lines for that character, and other models that include additive, dominance, and epistatic effects should be tested. If the model of no effect was rejected, I tested the simple additive model by repeating the above regressions after adding the column of additive coefficients to $\mathbf{M}$ (eqq. [2]–[3]). Rejection of the additive model indicates that dominance and/or epistatic effects also contribute to genetic divergence of the lines. Failure to reject the additive model indicates that divergence has been primarily at loci with additive effects. Lastly, if the simple additive model was rejected, the additive-plus-dominance model was tested. Rejection of the additive-plus-dominance model indicates that epistasis and/or linkage are contributing to genetic divergence of the lines.

Estimates of Gene Number

I used a biometric approach to estimate the effective number of genes contributing to differences between limnetics and benthics in the four morphological

characters. (I did not carry out this analysis for growth because of the smaller sample sizes for this trait.) The original method of Castle (1921) and Wright (1968) was intended for use with divergent inbred lines, but it was extended by Lande (1981) for use with wild populations. The method that I employed incorporates modifications suggested by Cockerham (1986) to correct for sampling variances in the estimates of parental populations. The basic formula is

$$\hat{n}_e = \frac{(\bar{z}_{\text{Plim}} - \bar{z}_{\text{Pben}})^2 - \text{var}(\bar{z}_{\text{Plim}}) - \text{var}(\bar{z}_{\text{Pben}})}{8\text{var}(s)}, \quad (5)$$

where $\bar{z}_i$ is the trait mean for line i, $\text{var}(\bar{z}_i)$ is the sampling variance of the trait mean for line i (the squared standard error), and $\text{var}(s)$ is the segregation variance.

I used three methods to calculate segregation variance. In the first two I did not use backcrosses in the calculations because of their small sample sizes relative to other lines and the possibility of slight heterosis in backcross lines (Hatfield 1995). I used the following alternative equations from Wright (1952, 1968):

$$\text{var}(s) = \text{var}(F_2) - \text{var}(F_1) \quad (6)$$

and

$$\text{var}(s) = \text{var}(F_2) - \{[2\text{var}(F_1) + \text{var}(P_{\text{lim}}) + \text{var}(P_{\text{ben}})]/4\}, \quad (7)$$

where $\text{var}(i)$ is the phenotypic variance among individuals in line i. Wright preferred the latter equation (7) because it partially corrects for any correlations between environmental variance and heterozygosity (Lande 1981). To correct for family effects on line variances, I used the pooled within-family variance estimates (Sokal and Rohlf 1981).

The third estimate of segregation variance used a maximum-likelihood method developed by Hayman (1960*b*) that incorporates information from all lines. The method is similar to the joint-scaling regressions described earlier for line means, and it uses the equations

$$\hat{\boldsymbol{c}} = (\mathbf{M}^T\mathbf{Y}^{-1}\mathbf{M})^{-1}\mathbf{M}^T\mathbf{Y}^{-1}\boldsymbol{v} \quad (8)$$

and

$$\hat{\boldsymbol{v}} = \mathbf{M}\hat{\boldsymbol{c}}, \quad (9)$$

where $\hat{\boldsymbol{c}}$ is the vector of variance components $\text{var}(z_{P1})$, $\text{var}(z_{P2})$, and $\text{var}(s)$; $\mathbf{M}$ is the matrix of coefficients from equations predicting line variances from parental and segregation variance (Hayman 1960*b*); $\boldsymbol{v}$ is the vector of observed line variances; and $\mathbf{Y}$ a diagonal matrix with variance elements, $2v_i^2/n$. Equations (8) and (9) are then iterated (substituting the elements of into $\mathbf{Y}$) until the elements of $\hat{\boldsymbol{v}}$ stabilize.

Wright's estimator assumes the following: additivity of gene effects, normality of data in each line, statistically independent data, and positive additive effects fixed in one parental line and negative additive effects in the other. The

resulting $\hat{n}_e$ should rarely exceed the recombination index, which in higher plants and animals is usually one to several times the haploid chromosome number (Lande 1981). Thus, $\hat{n}_e$ can be conservatively conceptualized as the number of chromosomes bearing loci affecting a trait; the haploid chromosome number in sticklebacks is 21 (Chen and Reisman 1970). If the assumptions are met, the method provides reasonably accurate estimates (Lande 1981), but it is unlikely that all assumptions will be met in wild (i.e., outbred) species. Failure to meet assumptions of this method generally bias the estimate downward, sometimes substantially (Zeng et al. 1990; Hill and Caballero 1992; Zeng 1992). The estimate is therefore often referred to as the minimum number of effective factors. Wright's estimator has several weaknesses that can be substantial under some circumstances (Cockerham 1986; Zeng et al. 1990; Hill and Caballero 1992; Zeng 1992), especially if one is looking for a precise estimate. For example, it is much more reliable when numbers of loci are small, when effects are constant across loci, and when parental line means differ by many standard deviations (Zeng 1992). Zeng (1992) has provided methods to help remove the bias in the estimator, but his modifications require genetic information not known for sticklebacks or most other species. (Gross estimates can be made for some of these parameters and will be addressed in the Discussion.) The original method therefore remains a valuable tool for initially exploring trait differences and for helping decide whether the traits are polygenic or controlled by only a few loci.

Lande (1981) provides formulas for bounding gene number estimates. However, if the assumptions of the model are incorrect, these bounds may be inflated or sometimes may not even include the true value. I therefore used the nonparametric bootstrapping technique (Efron and Tibshirani 1986) to obtain a more robust estimate of the lower bound on the gene number estimate. I carried out 1,000 bootstrap calculations of gene number (eq. [5]) in which each iteration used a random sample of morphological measurements from each line. These samples were drawn randomly with replacement and were of size equal to the original number of individuals measured in that line (see Efron and Tibshirani 1986 for details of the technique). Pooled within-family variances were reestimated for each iteration to recalculate segregation variance using equation (7). The result was a bootstrap estimate of the sampling distribution for the gene number estimates. The value corresponding to the fifth percentile of this sample yielded a lower bound to the 95% confidence interval of the gene number estimate. It should be noted, however, that when model assumptions are violated to different degrees (e.g., nonadditivity for some traits), such confidence intervals also do not necessarily bound the true values and are merely a good indicator.

RESULTS

Hybrid lines were intermediate between limnetics and benthics in every trait (table 1; fig. 1). The dotted lines in figure 1 join observed parental means and represent an a priori expectation of additive genetic effects: if limnetics and benthics have diverged primarily in genes with additive effects, then character means for all hybrid crosses should fall along this line. If the species have also

TABLE 1

CHARACTER MEANS ($\bar{z}$) AND VARIANCES (s^2) FOR THE SIX LINES

LINES	NUMBER OF INDIVIDUALS	NUMBER OF FAMILIES	GILL RAKER NUMBER		GILL RAKER LENGTH		PLATE NUMBER		PELVIC SPINE LENGTH		GROWTH RATE
			$\bar{z}$	s^2	$\bar{z}$	s^2	$\bar{z}$	s^2	$\bar{z}$	s^2	$\bar{z}$
Limnetic	33	16	24.156	(1.706)	.409	(.00317)	13.052	(1.137)	1.744	(.00198)	−.0306
Benthic	30	15	18.239	(1.061)	.064	(.00896)	1.250	(6.383)	.087	(.117)	.0299
F_1	88	21	21.029	(1.079)	.259	(.00657)	9.256	(.853)	1.487	(.0518)	.0300
F_2	122	31	21.177	(1.313)	.260	(.00857)	8.613	(4.912)	1.191	(.345)	.0034
Limnetic backcross	33	8	23.244	(1.161)	.296	(.00778)	11.338	(1.377)	1.557	(.0992)	−.0424
Benthic backcross	30	8	19.706	(.918)	.111	(.00849)	5.388	(5.748)	.990	(.172)	−.0322

NOTE.—Gill raker number and plate number are untransformed; gill raker length and pelvic spine length have been size corrected and are on a ln-scale; growth was corrected for rearing density (see the text). Means are means of family means, and variances are pooled within-family variances. (Note that since growth was measured on whole families, variances could not be calculated for this trait.) Sample sizes are for the four morphological traits. See the text for sample sizes of growth.

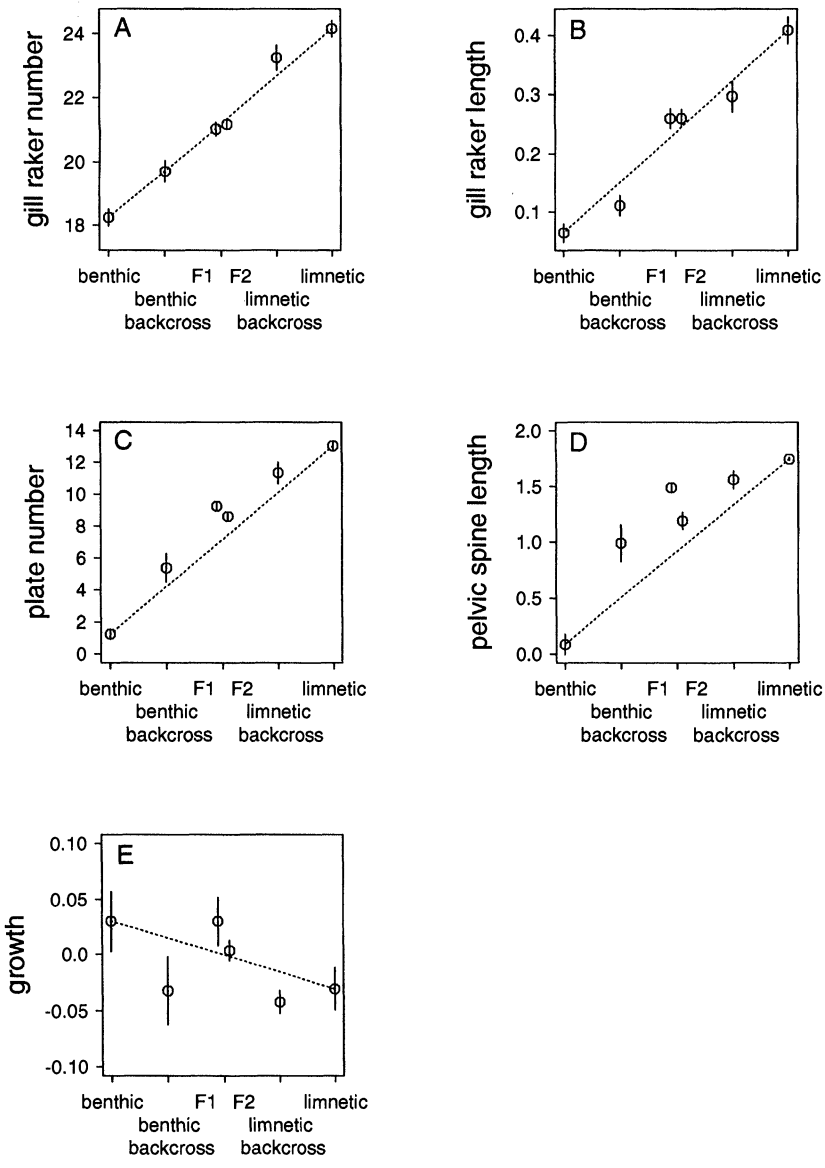

FIG. 1.—Observed character means and standard errors for the five traits measured in each of the six lines. A priori expectations of additive genetic effects are represented by the dotted lines drawn between parental means. If limnetics and benthics have diverged primarily in genes with additive effects, then character means for all hybrid crosses should fall along this line. Points for F_1 and F_2 hybrids have been displaced slightly to the left and right, respectively, to avoid overlap. Note that sample sizes differ among lines, so the error bars do not describe variance of the traits within lines; see table 1 for variances.

TABLE 2

PARAMETER ESTIMATES (SE) FROM JOINT-SCALING TESTS ON MEANS OF THE FIVE TRAITS

$\hat{a}$	A Gill Raker Number	A + D Gill Raker Length	A + D Plate Number	A + D Pelvic Spine Length	A Growth
$\hat{\mu}$	21.235 (.070)	.248 (.005)	8.394 (.108)	1.212 (.021)	−.0049 (.006)
$\hat{\alpha}$	−2.996 (.139)	−.173 (.010)	−5.551 (.214)	−.827 (.033)	.0412 (.013)
$\hat{\delta}$	. . .	.021 (.008)	1.006 (.124)	.287 (.023)	. . .

NOTE.—The joint-scaling tests were carried out for two genetic models, the additive-plus-dominance model (A + D) and the simple additive model (A). The model parameter estimates $\hat{\mu}$, $\hat{\alpha}$, and $\hat{\delta}$ are from vector $\hat{a}$ in equation (2).

diverged in alleles with dominance effects, then character means for hybrid crosses should all be displaced above or below the line—the displacement for F_1 hybrids should be double that for F_2 hybrids and backcrosses. The size of the displacement from the line is proportional to the degree of dominance.

The effects of additivity, dominance, and epistasis are apparent in figure 1. Five of six line means fall almost perfectly along the line in figure 1*A*, which suggests that gill raker number is an almost completely additive trait. In figure 1*C* and *D,* line means for F_1's are displaced from the line twice as much as those of F_2's and backcrosses, which illustrates clear dominance effects for plate number and pelvic spine length. Deviations from these two patterns in figure 1*B* suggest epistatic inheritance for gill raker length. Epistasis is also suggested by patterns in growth (fig. 1*E*), but large sampling errors for line means preclude such a conclusion. Note that additivity (i.e., the line drawn in the figures) remains a reasonably good fit for line means in all plots, with perhaps the exception of growth. This strongly suggests that the primary effects are additive for these traits.

Joint scaling uses least-squared regression to fit the best line to all points in figure 1 (i.e., not just a line between the parental means). The fit of the models to the five traits confirms the patterns apparent in figure 1. A model of no effect (i.e., $z_i = \mu + \epsilon_i$) was soundly rejected in all cases ($P \ll .01$), giving clear evidence of genetic divergence in all five characters. Further tests suggested that additive, dominance, and epistatic effects contribute to genetic divergence of limnetics and benthics for gill raker length (tables 2, 3). Rejection of the simple additive model indicated the presence of additive and dominance effects in plate number and pelvic spine length. A simple additive model was accepted for both gill raker number and growth.

I used the sum of squared residuals from each model (table 4) to quantify how good or poor the fit of the different models was. Despite the statistically poor fit of the additive model to four of the traits, table 4 shows that additivity still explains a large majority of the among-line variance. The exception is growth, which showed little difference between the two parental species.

Estimates of gene number (table 5) varied tremendously among the four morphological characters, though for each trait the three estimates of n_e were simi-

TABLE 3

GOODNESS-OF-FIT χ^2 TEST STATISTICS FROM JOINT-SCALING TESTS ON LINE MEANS OF THE FIVE CHARACTERS

Character	Additive Model (df = 4)	Additive-plus-Dominance (df = 3)
Gill raker number	2.58 NS	. . .
Gill raker length	10.29*	9.67*
Plate number	49.97***	1.86 NS
Pelvic spine length	82.93***	1.96 NS
Growth	9.24 NS	. . .

NOTE.—NS = not significant.
* $P < .05$.
*** $P < .001$.

TABLE 4

PERCENTAGE OF TOTAL VARIANCE EXPLAINED BY THE MODELS WHEN FIT TO THE MEANS OF THE FIVE CHARACTERS

Character	Percentage of Variance Explained by Additive Model	Additional Percentage of Variance Explained by Dominance Term	Residual Percentage of Variance
Gill raker number	98.6	<.1	1.4
Gill raker length	95.6	.3	4.1
Plate number	95.7	4.2	.1
Pelvic spine length	75.7	22.0	2.3
Growth	22.2	3.8	74.0

NOTE.—Residual variance is that left to be explained by epistasis and sampling error.

TABLE 5

ESTIMATES OF GENE NUMBER FOR THE FOUR MORPHOLOGICAL CHARACTERS, USING THREE ALTERNATIVE CALCULATIONS FOR SEGREGATION VARIANCE

Character	$\hat{n}_e1$	$\hat{n}_e2$	$\hat{n}_e3$
Gill raker number	17.49	50.02 (7.78)	na
Gill raker length*	7.42	6.58 (2.90)	4.95
Plate number*	4.14	6.44 (4.04)	11.95
Pelvic spine length*	1.16	1.18 (.97)	1.49

NOTE.—Estimation $\hat{n}_e1$ uses equation (6), $\hat{n}_e2$ uses equation (7), and $\hat{n}_e3$ uses maximum-likelihood estimates (see the text). Values in parentheses are the bootstrapped lower bounds of a 95% confidence limit for $\hat{n}_e2$.

* The simple additive model of genetic divergence was rejected for these traits.

lar. The maximum-likelihood estimate of segregation variance for gill raker number was negative, so $\hat{n}_e$ 3 was not calculated for this trait. Error in the estimates of trait variances commonly leads to negative estimates of segregation variance when the true number of genes is high (Zeng 1992). Therefore, accuracy in the estimates of trait variances becomes especially important at low values of segregation variance. Gene numbers for traits other than gill raker number may be underestimates since they did not fit an additive model (Zeng 1992). Nevertheless, the estimates are probably indicative since additive effects predominate (table 4). The confidence in these estimates is shown by the rough concordance among the three estimates and the bootstrap estimates of gene number (table 5). Ninety-five percent confidence intervals for gill raker number, gill raker length, and lateral plate number are bounded well away from 0, supporting an interpretation of polygenic control for these traits. The bootstrapped gene number estimates for pelvic spine length had a very tight distribution, which lends confidence to the low $\hat{n}_e$'s for this trait.

DISCUSSION

My results suggest that adaptive divergence between limnetic and benthic sticklebacks has taken place through a variety of genetic mechanisms specific to different traits. Additive effects, dominance, and epistasis contribute significantly to genetic divergence in gill raker length; additive effects and dominance explain divergence in plate number and pelvic spine length; and a simple additive model of genetic variance was sufficient to explain divergence for gill raker number and growth (tables 2, 3, 4). Although the simple additive model was rejected for three of the five traits, it explained >95% of total variance for three of the traits and >75% for a fourth (table 4). Statistical tests and residual variances for growth (tables 3, 4) suggest that sample sizes may have been insufficient to estimate line means adequately for this trait; the pattern among lines for this trait suggest further work is warranted.

The Castle-Wright method of gene number estimation gave a very broad range of estimates among the four morphological traits (table 5). Estimates suggested that differences between limnetics and benthics in three of the traits (gill raker number, gill raker length, and plate number) are determined by the action of more than four loci, whereas differences in pelvic spine length are determined primarily by only a single locus.

My expectation before carrying out these crosses had been that genetic divergence in these traits would be due to many loci acting additively, as is often the case with high- and low-selected lines in artificial selection experiments. This expectation seemed reasonable because the characters were previously shown to have high heritabilities (a measure of additive genetic variance) and because the species are of such recent divergence. The simple additive model did indeed explain most of the phenotypic variance among line means for each of the four morphological traits (fig. 1; table 4), which suggests that additive models may be adequate for modeling gross changes in quantitative characters during stickleback radiation. But although it is convention to apportion genetic variance in a

hierarchical order (additive, dominance, epistasis), the practice weights downward the likelihood of finding significant epistatic variation (Whitlock et al. 1995). Therefore, the finding of significant dominance and epistatic effects is likely robust, and we can conclude that phenotypic divergence is due to significant nonadditive gene action. I will first discuss the strengths and potential weaknesses in my data and then discuss the relevance of my findings to the genetics of adaptation and speciation.

Line Cross-Analyses

Line cross-analysis is well established, but the statistical models were developed under assumptions of Hardy-Weinberg and gametic phase equilibrium in the parental lines and an absence of maternal effects and linkage at those loci differing among populations (Mather and Jinks 1982; Lynch and Walsh 1997). McPhail's (1992) allozyme data showed that limnetics and benthics are each in Hardy-Weinberg equilibrium, but linkage disequilibrium was not estimated. The high chromosome number ($n = 21$) of sticklebacks (Chen and Reisman 1970) means that most pairs of loci will be unlinked, so linkage should not be a problem if loci are distributed throughout the genome. This cannot be assessed further without the development of linkage maps for these species.

Often line cross-analyses are based on very few replicate lines, though each replicate may contain many individuals. Consequently, a different set of parental lines could give quite different estimates. Even when several individuals make up the parental lines, they are usually mass mated so family effects are impossible to measure, even though among-family variance may make up a significant portion of the total observed variance. The major strength of the crossing design used in this study is that it used many full-sib families that were reared separately. Family effects on line variances could therefore be treated explicitly by using pooled within-family variance estimates (Sokal and Rohlf 1981). The conclusions from the joint-scaling tests should therefore be robust.

Gene Number Estimates

The biometric method of gene number estimation has several assumptions, so I will discuss the reliability of my estimates. First, the estimates are sensitive to hybrid breakdown and limited by the recombination index of the organism. There is no evidence in Paxton Lake sticklebacks of reduced fertilization, hatch success, or growth in F_1 or F_2 hybrids relative to limnetics and benthics and no evidence of reduced fecundity or parental care in F_1 hybrids (McPhail 1992; Hatfield 1995). However, there is some evidence of slightly lower hatch success and growth in the backcrosses (Hatfield 1995). Heterosis or underdominance are therefore unlikely to have affected the estimates significantly, especially since backcrosses were not used in two of the three estimates of segregation variance as a precaution. The recombination index of *Gasterosteus aculeatus* is not known, but since the haploid chromosome number is 21 (Chen and Reisman 1970), the index is probably two or three times this value and not likely a stringent restriction on estimates. If the loci affecting the traits measured here are not spread throughout the genome, however, linkage may cause substantial underes-

timation (Zeng et al. 1990). It is possible, for example, that the "single" locus estimated for pelvic spine length is in fact several tightly linked loci. We cannot properly evaluate the effects of linkage until fine-scale genetic maps are developed for sticklebacks.

Second, the method also assumes that divergence in mean phenotypes can be fit to an additive model, at least on some scale. Significant dominance and/or epistasis for four of the five characters violate this assumption, and standard transformations could not render additive the differences in mean phenotypes. The violation of this assumption suggests that all the estimates of gene number (table 5) are likely to be underestimates. Additive effects did explain most of the phenotypic variance among lines for each of the five traits (fig. 1; table 4), which suggests that violation of this assumption may not be substantial.

My goal in carrying out these analyses, however, was not to provide definitive estimates of gene number for these traits but to place some bounds on gene number, and in particular to understand whether differences in fitness-related traits are inherited polygenically. The large proportion of phenotypic variance explained by the additive models, the very high chromosome number of sticklebacks, and the bootstrap analysis all suggest that these are reliable as estimates of a minimum loci number.

Zeng (1992) provides methods to remove the bias of Wright's estimator. The key information needed to address the bias is the recombination index and the squared coefficient of variation of effects, neither of which are known for sticklebacks. When reasonable guesses are used (see Lynch and Walsh 1997), estimates are shifted upward, but the conclusions remain basically the same: polygenic inheritance for gill raker number, gill raker length, and plate number and single-locus inheritance for pelvic spine length. These qualitative conclusions therefore seem robust. Further work on growth differences is necessary before confident conclusions about inheritance patterns may be made for this trait.

Comparisons with Other Taxa

Four other recent studies have carried out genetic analysis of adaptive traits between natural populations or species (many others have studied traits in laboratory lines; see Lynch and Walsh 1997). In total, their results are similar to mine: nonadditive genetic variance was significant, and the number of loci varied among traits. Hard et al. (1992, 1993) examined divergence in diapause between northern and southern populations of the mosquito, *Wyeomyia smithii,* and found evidence for additive effects, dominance, and significant epistasis. Point estimates of gene number were large (14–19) and bounded well away from 0, although the authors stressed the low accuracy of these estimates given the evidence of significant nonadditive genetic effects. These may therefore be underestimates.

Three other studies investigated differences among wild species of the plant genus *Mimulus.* Macnair and Cumbes (1989) examined a wide array of floral and morphological differences, and Fenster et al. (1995) examined differences in floral development. Using line crosses, Macnair and Cumbes (1989) found evidence for both significant epistatic genetic variance and dominance effects (in addition to additivity) in a number of floral characters. Gene number estimates

varied from three to seven for some characters but were not reliably bounded above 0. The authors suggested control by many genes for some characters and by major genes for others. Fenster et al. (1995) used different species but also found evidence for epistasis and concluded that developmental traits in *Mimulus* are primarily polygenic. Using genetic mapping techniques, Bradshaw et al. (1995) obtained results suggesting only a few genes coding for the major ecological traits that confer reproductive isolation between a different pair of *Mimulus* species.

Implications for the Genetics of Adaptation and Speciation

As stated earlier, evidence seems to be building against the classical assumption that traits are polygenic and variation is entirely additive. However, I believe that Orr and Coyne's (1992) conclusions against polygenic inheritance are premature for several reasons. First, the authors were able to produce only eight studies that had looked at adaptive differences among natural populations. Though this highlights a pressing need for further research, at present these studies are too few in number on which to base significant generalizations. Second, the characters we choose for study affect our conclusions—some differences catch our eye more than others. Consider a character that is only present in one of two closely related species. Many genes may be required to build a complex character but only one to delete it; a single point mutation may be the only difference between two species even when the initial adaptation is polygenic. We must therefore be very cautious when extrapolating from genetic studies of species differences to the genetic properties of adaptations in general. Further criticisms revolve around possible shortcomings of the individual studies they reviewed.

My estimates of gene number offer a view that is nevertheless quite similar to that of Orr and Coyne (1992): some adaptive differences among species of stickleback are polygenic, while others may be explained by very few genes of large effect. The conclusion that we know little about the genetic basis of adaptive differences among populations will remain until there have been many studies of genetic differences among wild taxa. The current trend of mapping quantitative trait loci, or QTLs, may quickly rectify this gap in our knowledge.

Although it may be too early for generalizations regarding the number of loci for adaptive characters, the evidence of significant nonadditive genetic variation is growing quite large. My study can be added to a growing body of evidence (e.g., Jinks and Perkins 1969; Pooni et al. 1985; Macnair and Cumbes 1989; Hard et al. 1992, 1993; Cabot et al. 1994; Davis et al. 1994; Lai et al. 1994; Long et al. 1995; Mckay 1995) demonstrating that many characters do not fit a simple additive model. The implications of this finding are less clear: though these effects are significant in statistical models, their biological significance remains poorly understood.

One possible lesson is that it tells us about the evolution of reproductive isolation and what I would call "ecological underdominance." When benthic and limnetic sticklebacks are crossed, there is little or no measurable difference in physiological competence relative to the parental species; hybrids show neither positive nor negative heterosis in the laboratory (McPhail 1984, 1992, 1994;

Hatfield 1995). However, the picture is very different in the wild: when limnetics, benthics, and hybrids are enclosed in either of the two main habitats in the wild, the hybrids do significantly worse overall than the parental species do (Schluter 1995; Hatfield 1995). This reduced vigor (i.e., underdominance) is directly related to the specific modes of inheritance of structures such as gill rakers. Hybrids are selected against because they are morphologically intermediate and therefore unable to exploit efficiently either of the main feeding habitats (Schluter 1993). It is important to understand the genetic basis of quantitative traits because different modes of inheritance of feeding apparatus or armor will lead to different patterns of hybrid fitness in the wild through ecologically driven natural selection. For example, dominance in armor characteristics would tend to give excessive armor (i.e., greater than that expected if the trait were purely additive) to otherwise benthic-like hybrids (e.g., benthic backcrosses), making them more susceptible to insect predators. It is worth emphasizing that these ecological effects have a genetic basis. Ecological underdominance is not a phenomenon restricted to sticklebacks. For example, fitness of interspecific hybrids in Darwin's finches is determined by morphology and relative resource abundances (Grant and Grant 1993), and morphology has a strong genetic basis (Boag and Grant 1978). It would be interesting to know whether ecological underdominance generally precedes the more commonly and more easily studied effects such as sterility or inviability.

Adaptation to divergent resources confers the only known postzygotic isolation between limnetic and benthic sticklebacks (Hatfield 1995). Because the morphological characters that I examined play a role in acquiring these resources, they indirectly confer reproductive isolation. It is too early to say whether these are truly speciation genes, but they do currently confer some postzygotic isolation. In our studies of reproductive isolation in sticklebacks, we have partitioned selection against hybrids into developmental and ecological components (McPhail 1984, 1992, 1994; Schluter and McPhail 1992; Schluter 1993, 1995; Hatfield 1995) and have now explored the genetic basis for some quantitative traits. The next step should be to partition ecological selection against hybrids into additive, dominance, and epistatic effects. This could be done by manipulating the individual traits and examining their effects on fitness in the wild. This should present a very interesting picture of the evolution of reproductive isolation.

ACKNOWLEDGMENTS

I thank T. Price for the initial suggestion to estimate gene number; S. Cotton, L. Hummelbrunner, and S. Wu for help with fish rearing; and J. D. McPhail and D. Schluter for many lively and helpful discussions along the way. E. D. Brodie, C. J. Foote, S. B. Heard, J. D. McPhail, D. Schluter, J. N. M. Smith, M. J. Wade, and three anonymous reviewers provided thoughtful criticism of the manuscript. I gratefully acknowledge the use of unpublished chapters on genetic analysis by M. Lynch and J. B. Walsh. The research was funded by grants from the Natural Science and Engineering Research Council (Canada) to D. Schluter.

LITERATURE CITED

Baggerman, B. 1957. An experimental study on the timing of breeding and migration in the three-spined stickleback, *Gasterosteus aculeatus* L. Archives Néerlandaises de Zoologie 12:105–318.

Bell, M. A., and Foster, S. A. 1994. Introduction to the evolutionary biology of the threespine stickleback. Pages 1–27 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Bell, M. A., and T. R. Haglund. 1978. Selective predation of threespine sticklebacks *Gasterosteus aculeatus* by garter snakes. Evolution 32:304–319.

Boag, P. T., and P. R. Grant. 1978. Heritability of external morphology in Darwin's finches. Nature (London) 274:793–794.

Bradshaw, H. D., Jr., S. M. Wilbert, K. G. Otto, and D. W. Schemske. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). Nature (London) 376:762–765.

Cabot, E. L., A. W. Davis, N. A. Johnson, and C.-I. Wu. 1994. Genetics of reproductive isolation in the *Drosophila simulans* clade: complex epistasis underlying hybrid male sterility. Genetics 137:175–189.

Castle, W. E. 1921. An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. Science (Washington, D.C.) 54:223.

Cavalli, L. L. 1952. An analysis of linkage in quantitative inheritance. Pages 135–144 *in* E. C. R. Reeve and C. H. Waddington, eds. Quantitative inheritance. Her Majesty's Stationery Office, London.

Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. Evolution 44:520–538.

Chen, T.-R., and H. M. Reisman. 1970. A comparative chromosome study of the North American species of sticklebacks (Teleostei: Gasterosteidae). Cytogenetics 9:321–332.

Cheverud, J. 1984. Quantitative genetics and developmental constraints on evolution by selection. Journal of Theoretical Biology 110:155–171.

Cockerham, C. C. 1954. An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present. Genetics 39:859–882.

———. 1986. Modifications in estimating the number of genes for a quantitative character. Genetics 114:659–664.

Davis, A. W., E. G. Noonburg, and C.-I. Wu. 1994. Evidence for complex genic interactions between conspecific chromosomes underlying female sterility in the *Drosophila simulans* clade. Genetics 137:191–199.

Day, T., J. Pritchard, and D. Schluter. 1994. Ecology and genetics of phenotypic plasticity: a comparison of two sticklebacks. Evolution 48:1723–1734.

Efron, B., and R. Tibshirani. 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Statistical Science 1:54–77.

Fenster, C. B., P. K. Diggle, S. C. H. Barrett, and K. Ritland. 1995. The genetics of floral development differentiating two species of *Mimulus* (Scrophulariaceae). Heredity 74:258–266.

Fisher, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Transactions of the Royal Society of Edinburgh 52:399–433.

Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121:124–138.

Grant, B. R., and P. R. Grant. 1993. Evolution of Darwin's finches caused by a rare climatic event. Proceedings of the Royal Society of London B, Biological Sciences 251:111–117.

Hagen, D. W. 1973. Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculeatus.* Heredity 30:303–312.

Hagen, D. W., and L. G. Gilbertson. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. Evolution 26:32–51.

Hard, J. J., W. E. Bradshaw, and C. M. Holzapfel. 1992. Epistasis and the genetic divergence of photoperiodism between populations of the pitcher-plant mosquito, *Wyeomyia smithii.* Genetics 131:389–396.

———. 1993. The genetic basis of photoperiodism and its evolutionary divergence among populations of the pitcher-plant mosquito, *Wyeomyia smithii.* American Naturalist 142:457–473.

Hatfield, T. 1995. Speciation in sympatric sticklebacks: hybridization, reproductive isolation and the maintenance of diversity. Ph.D. diss. University of British Columbia, Vancouver.

———. In press. Fluctuating asymmetry and reproductive isolation between two sticklebacks. Environmental Biology of Fishes.

Hayman, B. I. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12:371–390.

———. 1960*a*. Maximum likelihood estimation of genetic components of variation. Biometrics 16: 369–381.

———. 1960*b*. The separation of epistatic from additive and dominance variation in generation means. II. Genetica 31:133–146.

Hill, W. G., and A. Caballero. 1992. Artificial selection experiments. Annual Review of Ecology and Systematics 23:287–310.

Jinks, J. L., and J. M. Perkins. 1969. Detection of linked epistatic genes for a metrical trait. Heredity 24:465–475.

Kempthorne, O. 1954. The correlation between relatives in a random mating population. Proceedings of the Royal Society of London B, Biological Sciences 143:103–113.

Kliewer, E. V. 1970. Gill raker variation and diet in lake whitefish, *Coregonus clupeaformis* in northern Manitoba. Pages 147–165 *in* C. C. Lindsey and C. S. Woods, eds. Biology of coregonid fishes. University of Manitoba Press, Winnipeg.

Lai, C., R. F. Lyman, A. D. Long, C. H. Langley, and T. F. C. Mackay. 1994. Naturally occurring variation in bristle number and DNA polymorphisms at the scabrous locus of *Drosophila melanogaster*. Science (Washington, D.C.) 266:1697–1702.

Lande, R. 1980*a*. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203–215.

———. 1980*b*. Genetic variation and phenotypic evolution during allopatric speciation. American Naturalist 116:463–479.

———. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541–553.

———. 1982. A quantitative genetic theory of life history evolution. Ecology 63:607–615.

Long, A. D., S. L. Mullaney, L. A. Reid, J. D. Fry, C. H. Langley, and T. F. C. Mackay. 1995. High resolution mapping of genetic factors affecting abdominal bristle number in *Drosophila melanogaster*. Genetics 139:1273–1291.

Lynch, M., and J. B. Walsh. 1997. Quantitative genetics: biology and estimation. Sinauer, Sunderland, Mass.

Macnair, M. R., and Q. J. Cumbes. 1989. The genetic architecture of interspecific variation in *Mimulus*. Genetics 122:211–222.

Magnuson, J. J., and J. G. Heitz. 1971. Gill raker apparatus and food selectivity among mackerels, tunas, and dolphins. Fisheries Bulletin 69:361–370.

Mather, K., and J. L. Jinks. 1982. Biometrical genetics: the study of continuous variation. 3d ed. Chapman & Hall, New York.

Mckay, T. F. C. 1995. The genetic basis of quantitative variation: numbers of sensory bristles of *Drosophila melanogaster* as a model system. Trends in Genetics 11:464–470.

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62:1402–1408.

———. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. Canadian Journal of Zoology 70:361–369.

———. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. Canadian Journal of Zoology 71:515–523.

———. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. Pages 399–437 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Orr, H. A., and J. A. Coyne. 1992. The genetics of adaptation: a reassessment. American Naturalist 140:725–742.

Pooni, H. S., J. L. Jinks, and J. F. Detoledo. 1985. Predicting and observing the properties of 2nd cycle hybrids using basic generations and inbred line $\times$ F_1 crosses. Heredity 54:121–129.

Reimchen, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus:* an adaptation to predators? Canadian Journal of Zoology 58:1232–1244.

———. 1988. Inefficient predators and prey injuries in a population of giant stickleback. Canadian Journal of Zoology 66:2036–2044.

———. 1992. Injuries and survival of *Gasterosteus* from attacks by a toothed predator (*Oncorhyncus*) and some implications for the evolution of lateral plates. Evolution 46:1224–1230.

———. 1994. Predators and morphological evolution in threespine stickleback. Pages 240–276 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford.

Reist, J. D. 1980. Predation upon pelvic phenotypes of brook stickleback, *Culea inconstans,* by selected invertebrates. Canadian Journal of Zoology 58:1253–1258.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rose, M. R. 1985. Life history evolution with antagonistic pleiotropy and overlapping generations. Theoretical Population Biology 28:42–358.

Sanderson, S. L., J. J. Cech, and M. R. Patterson. 1991. Fluid dynamics in suspension-feeding blackfish. Science (Washington, D.C.) 251:1346–1348.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape and habitat use efficiency. Ecology 74:699–709.

———. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. Science (Washington, D.C.) 266:798–801.

———. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82–90.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

———. 1993. Character displacement and replicate adaptive radiation. Trends in Ecology & Evolution 8:197–200.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2d ed. W. H. Freeman, New York.

Statistical Sciences. 1991. S-plus. StatSci, Seattle, Wash.

Taylor, E. B., J. D. McPhail, and D. Schluter. In press. History of ecological selection in sticklebacks: uniting experimental and phylogenetic approaches. *In* T. J. Givnish and K. J. Sytsma, eds. Molecular evolution and adaptive radiation. Cambridge University Press, Cambridge.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–523.

Whitlock, M. C., P. C. Phillips, F. B.-G. Moore, and S. J. Tonsor. 1995. Multiple fitness peaks and epistasis. Annual Review of Ecology and Systematics 26:601–629.

Wooton, R. J. 1973. The effect of size of food ration on egg production in the female three-spined stickleback, *Gasterosteus aculeatus* L. Journal of Fish Biology 5:89–96.

Wright, S. 1952. The genetics of quantitative variability. Pages 5–41 *in* E. C. R. Reeve and C. H. Waddington, eds. Quantitative inheritance. Her Majesty's Stationery Office, London.

———. 1968. Evolution and the genetics of populations. Vol. 1. Genetic and biometric foundations. University of Chicago Press, Chicago.

Zeng, Z.-B. 1992. Correcting the bias of Wright's estimates of the number of genes affecting a quantitative character: a further improved method. Genetics 131:987–1001.

Zeng, Z.-B., D. Houle, and C. C. Cockerham. 1990. How informative is Wright's estimator of the number of genes affecting a quantitative character? Genetics 126:235–247.

Zyuganov, V. V. 1989. The discovery of reproductively isolated parapatric forms of the ninespine stickleback, *Pungitius pungitius,* with and without a pelvic girdle. Soviet Journal of Fish Biology 1989:134–142.

Associate Editor: J. Bruce Walsh