JOURNAL OF Evolutionary Biology

doi: 10.1111/j.1420-9101.2011.02330.x

Quantitative genetic inheritance of morphological divergence in a lake–stream stickleback ecotype pair: implications for reproductive isolation

D. BERNER*, R. KAEUFFER†, A.-C. GRANDCHAMP*, J. A. M. RAEYMAEKERS‡, K. RÄSÄNEN§ & A. P. HENDRY†

*Zoological Institute, University of Basel, Basel, Switzerland
†Redpath Museum & Department of Biology, McGill University, Montreal, QC, Canada
‡Laboratory of Animal Diversity and Systematics, Katholieke Universiteit Leuven, Leuven, Belgium
§Department of Aquatic Ecology, ETH-Zurich, Institute of Integrative Biology and Eawag, Duebendorf, Switzerland

Keywords:

additive genetic variance;
dominance;
epistasis;
foraging morphology;
Gasterosteus aculeatus;
genetic architecture;
geometric morphometrics;
hybrid;
line cross analysis.

Abstract

Ecological selection against hybrids between populations occupying different habitats might be an important component of reproductive isolation during the initial stages of speciation. The strength and directionality of this barrier to gene flow depends on the genetic architecture underlying divergence in ecologically relevant phenotypes. We here present line cross analyses of inheritance for two key foraging-related morphological traits involved in adaptive divergence between stickleback ecotypes residing parapatrically in lake and stream habitats within the Misty Lake watershed (Vancouver Island, Canada). One main finding is the striking genetic dominance of the lake phenotype for body depth. Selection associated with this phenotype against first- and later-generation hybrids should therefore be asymmetric, hindering introgression from the lake to the stream population but not *vice versa*. Another main finding is that divergence in gill raker number is inherited additively and should therefore contribute symmetrically to reproductive isolation. Our study suggests that traits involved in adaptation might contribute to reproductive isolation qualitatively differently, depending on their mode of inheritance.

Introduction

Speciation probably often starts as a result of adaptation to ecologically different environments (Dobzhansky, 1951; Schluter, 2000; Coyne & Orr, 2004; Rundle & Nosil, 2005; Sobel *et al.*, 2010). A component of reproductive isolation that might be particularly important during this process is selection against hybrids. That is, even in the face of dispersal across habitat boundaries and associated interbreeding, gene flow can be restricted if the phenotypes of first- and later-generation hybrids perform poorly relative to better-adapted resident phenotypes (Arnold, 1997; Schluter, 2000).

One factor that will influence the contribution of selection against hybrids to reproductive isolation is the extent to which phenotypic shifts between habitats are genetically based as opposed to being environmentally induced (Arnold, 1997; Crispo, 2008). That is, if habitat-related differences in traits under divergent selection are primarily caused by phenotypic plasticity, hybrid offspring derived from migrants to foreign habitats may express resident phenotypes and hence perform well, facilitating gene flow across habitats (Thibert-Plante & Hendry, 2011). On the other hand, if adaptive divergence is genetically based, hybrid phenotypes and their performance in a given habitat relative to pure resident phenotypes will depend on the underlying quantitative genetic architecture and the associated mode of inheritance (Arnold & Hodges, 1995; Rieseberg, 1995; Arnold, 1997; Barton, 2001). For example, if adaptive divergence is due to genetic factors whose total net effect is primarily additive, hybrids will be phenotypically intermediate between the pure types and hence be selected against in both habitats (Barton & Hewitt, 1985; Schluter, 2000; Rundle & Nosil, 2005). By contrast, if adaptive divergence is due to genetic factors whose combined net effect

Correspondence: Daniel Berner, Zoological Institute, University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland.
Tel.: +41 (0) 61 267 03 28; fax: +41 (0) 61 267 03 01;
e-mail: daniel.berner@unibas.ch

is primarily dominant, hybrids will more closely resemble one of the parent types than the other. This renders the strength of selection against hybrids, and hence, the opportunity for introgression, asymmetric (i.e. habitat-dependent). These patterns might be further complicated by epistatic effects (interactions among loci) whose consequences in an ecological context are difficult to predict.

Understanding the role of selection against hybrids in speciation therefore benefits from information on the quantitative genetic basis of traits known or suspected to be involved in ecologically based reproductive isolation (Arnold & Hodges, 1995; Hatfield, 1997; Czesak *et al.*, 2004; Fritz *et al.*, 2006; Barson *et al.*, 2007; Rego *et al.*, 2007; Fuller, 2008). Our goal is to provide such information for key morphological traits in an emerging system for studying incipient speciation – threespine stickleback fish (*Gasterosteus aculeatus*) residing in lake and stream habitats.

Threespine stickleback occur in contiguous lake and stream habitats in many watersheds that were colonized independently after the last glacial retreat (Hagen & Gilbertson, 1972; Reimchen *et al.*, 1985; Lavin & McPhail, 1993; Thompson *et al.*, 1997; Reusch *et al.*, 2001; Hendry & Taylor, 2004; Aguirre, 2009; Berner *et al.*, 2009, 2010). These two habitats are ecologically different in that lakes typically provide opportunities for foraging on both limnetic prey (zooplankton in the open water) and benthic prey (macroinvertebrates on the substrate), whereas streams provide almost exclusively the latter (Berner *et al.*, 2008, 2009). This ecological difference between lakes and streams generates divergent selection driving adaptive divergence in stickleback foraging traits. Moreover, adaptive divergence between lake and stream populations frequently, although not always, goes hand in hand with reproductive isolation (Berner *et al.*, 2009, 2010). As a result, lake–stream stickleback often form well-differentiated ecotype (or incipient species) pairs, sometimes even when occurring in close proximity without physical barriers to dispersal.

The morphological traits that display the most consistent divergence between lake and stream ecotypes, and that are targeted by resource-based selection, include body shape and gill raker number. For body shape, stickleback in lakes have shallower bodies than do stickleback in streams (Hagen & Gilbertson, 1972; Reimchen *et al.*, 1985; Lavin & McPhail, 1993; Hendry *et al.*, 2002; Hendry & Taylor, 2004; Aguirre, 2009; Berner *et al.*, 2009), which presumably improves swimming performance when foraging on zooplankton in the open water (Webb, 1984; Blake, 2004; Hendry *et al.*, 2011). By contrast, the deeper bodies of stream stickleback presumably improve manoeuvrability and hence facilitate foraging on complex bottom substrates (Walker, 1997; Hendry *et al.*, 2011). In addition to the divergence in body shape, lake stickleback display a greater number of gill rakers (bony tubercles on the first branchial arch) than do stream ecotypes (Hagen & Gilbertson, 1972; Gross & Anderson, 1984; Lavin & McPhail, 1993; Hendry & Taylor, 2004; Berner *et al.*, 2009). This divergence presumably promotes capture and handling efficiency of prevailing prey types in the two habitats (Schluter, 1993; Robinson, 2000).

Common-garden experiments indicate that lake–stream divergence in stickleback body shape and gill raker number has a substantial genetic basis, at least in the one system (Misty) where such studies have been performed (Lavin & McPhail, 1993; Sharpe *et al.*, 2008; Hendry *et al.*, 2011). Further, field transplant experiments suggest that lake–stream divergence leads to superior performance of stickleback in their home environments, and hence likely causes selection against migrants (Hendry *et al.*, 2002). Selection against first- and later-generation hybrids, however, would depend on the relative contribution of additivity, dominance and epistasis to ecotype divergence.

A powerful approach to obtaining this information on genetic architecture is the analysis of line crosses (Hayman, 1958; Mather & Jinks, 1982; Lynch & Walsh, 1998). Two such studies have been performed for threespine stickleback: one for marine and freshwater population pairs (Schluter *et al.*, 2004) and one for sympatric limnetic-benthic species (Hatfield, 1997). Together, these studies suggest an entirely additive basis to differences in body shape and gill raker number. Whether this holds for lake–stream stickleback is unknown. We therefore here present a line cross analysis of body shape and gill raker number inheritance for the lake–inlet stream stickleback ecotype pair residing parapatrically within the Misty watershed on Vancouver Island, British Columbia, Canada.

Materials and methods

Source populations and laboratory lines

The stickleback used for this study originate from three consecutive generations raised from 2004 to 2008 under controlled laboratory conditions at McGill University, Montreal, Canada. The first generation included eight pure families from Misty Lake and four pure families from the inlet stream flowing into Misty Lake. (Fewer families were used for the inlet because fewer gravid fish were available at the time of sampling.) These families were created in June 2004 by artificially crossing field-caught fish from each of the two sites: Misty Lake site 1 and inlet stream site 4 (Delcourt *et al.*, 2008; sites are described in Moore & Hendry, 2005). Each cross used unique individuals only, and offspring from each family were split across 20–100 L aquaria to achieve a density of approximately 25 fish per 100 L. The juveniles were fed live *Artemia* nauplii for 6 weeks, and then frozen chironomid larvae. Initial 'summer' laboratory conditions were 18 °C with a 16 : 8 h day : night photoperiod.

After approximately 1 year, we simulated 'winter' conditions by reducing the temperature to 16 °C and the photoperiod to 8 : 16 h day : night for 2.5 months. Thereafter, summer conditions were re-established to stimulate reproduction, and the second generation was created in winter 2005/2006. This generation included pure lake crosses (five families), pure stream crosses (six families), and their F1 hybrids (seven families). The hybrid crosses included both reciprocal parental combinations (lake male × stream female and stream male × lake female). Each cross again used unique individuals, and sib mating was not allowed. The aforesaid protocol was then repeated to create the third generation in the spring of 2007, this one consisting of pure lake and stream crosses (one family each), seven F1 hybrid crosses, five F2 hybrid crosses, nine lake backcrosses and five stream backcrosses. All reciprocal parental combinations were included in the hybrids and backcrosses. The third generation was terminated in the spring of 2008.

All fish from each generation were measured (as described in the following paragraphs) at approximately 1 year of age, thus reaching typical adult body sizes seen in nature. Overall, the study included 792 individuals from 58 families. A few crosses yielded data for males only (one family), or for females only (three families) (see Table 1 for a summary of families per line type and sex). Generally, we measured a minimum of five individuals per sex and family combination, although sometimes less than five individuals were available (male average = 5.3, SD = 3.8; female average = 8.8, SD = 5.2).

Phenotypic measurements

Stickleback were killed with an overdose of MS-222, immediately placed on their right side in natural position on a standard background with a reference scale, and then photographed with a digital camera. Fine pins were used to indicate landmarks otherwise difficult to locate on the photographs. The specimens were subsequently sexed by dissection, and stored in 95% ethanol.

Given that the use of geometric morphometrics to analyse overall body shape is now standard in work on stickleback and many other species, we also started with this approach. We used tpsDig (Rohlf, 2001) to place the same 16 landmarks as in Berner *et al.* (2010) on each photograph. Next, we used tpsRelw (Rohlf, 2001) to compute the consensus landmark configuration for each sex-by-family combination (N = 112). All consensus configurations were then analysed together in tpsRelw to obtain the weight matrix (summarizing uniform and localized components of shape variation), and to extract its principal components (relative warps). The first relative warp (RW1) captured a large proportion (43.5%) of the total shape variation among the consensus configurations and displayed strong divergence between the lake and stream ecotypes. RW2 accounted for 17.7% of the total variation and captured some sexual dimorphism, but was only weakly associated with ecotype. RW3 (10.8%) captured bending of specimens during placement for photographs and hence was biologically irrelevant. All subsequent RWs accounted for less than 8.9% of the variation and also showed no ecotype association. RW1 thus emerged as the main axis of body shape divergence between the ecotypes, consistent with previous work (Berner *et al.*, 2009, 2010; Hendry *et al.*, 2011). We therefore restricted our analysis of geometric morphometric body shape inheritance on RW1.

Because the aforesaid shape analysis extracted RW1 scores from consensus configurations of unit centroid size, it was not possible to partial out size-related shape variation. We therefore also performed an additional analysis where RW1 was computed from the individual (N = 792) landmark configurations. Individual RW1 scores were then regressed against centroid size, and the residuals used to compute sex-by-family averages. The analysis of inheritance for this main axis of size-independent body shape divergence produced results very similar to the aforesaid analysis of RW1, supporting identical conclusions. The analysis based on size-independent RW1 is therefore not presented.

In addition to RW1, we quantified more specific aspects of body shape through two univariate linear distance measurements taken from each photograph: body depth and snout length. (We also examined other univariate traits but do not report them here because they did not show divergence between ecotypes, and do not have as clear a functional interpretation.) We here defined univariate 'body depth' as the Euclidean distance between the landmarks located at the anterior insertion of the second dorsal spine and the pelvic spine (see Berner *et al.*, 2009 for an illustration). Univariate 'snout length' was measured as the distance between the landmarks at the tip of the upper jaw and at the posterior edge of the eye. We included these traits in our analysis for consistency with earlier work where body shape

Table 1 Number of replicate families for each line type and sex (pooled across generations), along with the coefficients for composite genetic effects used in the models of trait inheritance. The first and second coefficients represent additive [a] and dominance [d] genetic effects. The last three coefficients reflect digenic epistasis (i.e. additive–additive [aa], additive–dominance [ad], and dominance–dominance [dd] interaction between two loci). The intercept coefficient is not shown but was implicit in all models.

Line type	N (m/f)	[a]	[d]	[aa]	[ad]	[dd]
Stream	11/10	−1	0	1	0	0
Stream backcross	4/5	−0.5	0.5	0.25	−0.25	0.25
F1 hybrid	13/14	0	1	0	0	1
F2 hybrid	5/5	0	0.5	0	0	0.25
Lake backcross	8/9	0.5	0.5	0.25	0.25	0.25
Lake	14/14	1	0	1	0	0

variation was studied using similar linear distance traits (e.g. Reimchen *et al.*, 1985; Lavin & McPhail, 1993; Hendry *et al.*, 2002; Berner *et al.*, 2008). In addition, these two traits isolated the two key aspects of RW1 (see Results). Both body depth and snout length were size-corrected by taking an analysis of covariance approach (Reist, 1985; Berner, 2011) with centroid size as a covariate (calculated from individual landmark configurations in tpsRelw). The size-corrected values were used to compute sex-by-family means.

Using the preserved specimens, gill raker number was counted on the ventral bone of the first gill arch (as in Berner *et al.*, 2008) under a stereomicroscope at 45× magnification. Average values were then calculated for each sex-by-family combination.

Prior to line cross analysis, we used the line types available from multiple years (pure lines, F1 hybrids) to test for consistency among the laboratory generations in RW1, body depth, snout length and gill raker number. All these phenotypes proved highly consistent among generations (details not presented), indicating that if environmental maternal effects occur in these traits, they are very weak. In addition, we tested for differences between the reciprocal parental combinations within the F1 hybrids and backcrosses, but found no indication of such effects in any trait (details not presented). For each line cross type, we therefore combined data from different generations and parental combinations.

Finally, we considered the possibility of genetic coupling among traits due to pleiotropy or physical linkage among loci. This was examined qualitatively by estimating the phenotypic correlation between the body shape–related traits (RW1, body depth and snout length) and gill raker number, and between univariate body depth and snout length. We here used only F2 hybrids, the line type with the weakest gametic phase disequilibrium among loci derived from the lake and stream population. These analyses found no evidence for genetic coupling between traits (details not presented), thus justifying our univariate analytical approach.

Line cross analysis

We performed line cross analysis to determine the extent to which additive gene action, dominance and epistatic gene interactions explained deviations of observed line means from the means predicted for a theoretical population with random segregation of genetic factors (i.e. a F_∞ population deriving from an initial cross between the pure ecotypes; Lynch & Walsh, 1998). Line cross analysis was carried out by fitting the data to a hierarchy of increasingly complex genetic models.

For all traits, we started with a basic genetic model including only composite additive gene action [a] (i.e. additive effects summed over all loci) and sex as factors. Under purely additive inheritance and without sampling error, F1 and F2 hybrid means are expected to lie exactly between the pure ecotype means, and backcross means are expected to be exactly intermediate between the F1 hybrid and the corresponding pure ecotype means. [Note that additivity is the first composite genetic effect entered in line cross analysis models (Mather & Jinks, 1982; Lynch & Walsh, 1998).] Sex was added to account for possible sex-linked genetic effects (the sex-additive interaction was always unimportant [$P \geq 0.211$] and hence ignored). For snout length, the base model revealed strong sexual dimorphism ($F_{1,109} = 134.9$, $P < 0.0001$) but surprisingly indicated no difference between the cross lines ($F_{1,109} = 0.051$, $P = 0.822$; a similar result was obtained when using data from the pure lines only). Snout length was therefore not analysed further, but the data are visualized in Fig. A1. For gill raker number, the initial base model indicated no effect of sex. We here therefore pooled male and female individuals to calculate family means, yielding a reduced data set of $N = 58$, and specified a new base model containing only additive gene action as factor.

The next higher-level model additionally included a composite dominance parameter [d], accounting for dominance of one ecotype over the other summed across all loci. The final and most complex model additionally incorporated composite digenic epistasis (i.e. the net effects of additive–additive [aa], additive–dominance [ad] and dominance–dominance [dd] interactions between two loci) (Hayman, 1958; Mather & Jinks, 1982; Lynch & Walsh, 1998), which would explain significant deviation from the additive or dominance expectations. We made no attempt to separate the different epistatic components because we considered statistical power insufficient for this purpose. The coefficients for the model parameters were specified as in Mather & Jinks (1982) and are summarized in Table 1.

Each model's explanatory power was evaluated using an information-theoretical approach (a frequentist approach using chi-squared likelihood ratio tests led to very similar results, which are therefore not presented). This approach searched for the model that explained the most variance among line means while being penalizing for the number of model parameters. We extracted the Akaike information criterion (AIC) for each model and used it to compute the second-order information criterion (AIC_c) recommended for relatively small sample sizes (Burnham & Anderson, 2002). (AIC_c and AIC differed only very slightly and supported the same conclusions.) The model with the lowest AIC_c was considered the best model unless its AIC_c was only 0–2 units below that of a simpler (more parsimonious) model (Burnham & Anderson, 2002). For RW1, body depth and gill raker number, we also performed the line cross analysis for male and female data separately ($N = 55$ and 57). All statistics and plotting were performed by using the R language (R Development Core Team, 2010). All data used for this study are available on the Dryad digital repository (doi: 10.5061/dryad.6vq04).

Results

The dominant axis of geometric morphometric shape variation among the sex-by-family consensus configurations (RW1) primarily captured shifts in overall body depth and snout length (Fig. 1). Along this axis, the lake ecotype displayed a more slender body and a shorter snout compared with the stream ecotype, driving a highly significant additive genetic term in the base model ($F_{1,109} = 163$, $P < 0.0001$). Sexual dimorphism was also present ($F_{1,109} = 124$, $P < 0.0001$) and roughly similar in magnitude to the ecotype difference. Females exhibited more slender bodies and shorter snouts than males. The fit of the base model could be greatly improved by adding a dominance parameter (Table 2). Here, genetic factors associated with the lake ecotype proved dominant on average over the stream ecotype (Fig. 1). Adding a sex-dominance interaction further increased model fit slightly, reflecting that dominance gene action was more pronounced in males. Best model fit was achieved by adding the epistatic components. Sex-specific analyses confirmed dominance gene action in both sexes but suggested that epistasis was limited to males (details not presented).

The univariate analysis of body depth produced results that were similar in some respects and different in others to the shape analysis based on RW1. As for RW1, we here found very a strong ecotype difference (deeper bodies in stream fish), yielding a highly significant additive genetic term in the base model ($F_{1,109} = 115$, $P < 0.0001$) (Fig. 2). Sexual dimorphism, however, was very weak, albeit significant ($F_{1,109} = 7.3$, $P = 0.008$). Model fit could be greatly increased by adding the dominance term (Table 2), but there was no indication that dominance differed between the sexes. Dominance was strong, with both F1 and F2 hybrids and even some stream backcrosses closely resembling the lake phenotype. Deviations from the dominance model, however, were still substantial so that adding epistasis increased model fit further. Sex-specific analyses (not shown) produced very similar results.

Lake ecotypes had more numerous gill rakers than their stream counterparts, as reflected in the highly significant additive term in the base model ($F_{1,109} = 75$, $P < 0.0001$) (Fig. 3). Sex had no influence on gill raker number ($F_{1,109} = 0.48$, $P = 0.489$). The sexes were therefore pooled for the estimation of family means, leading to a new base model containing only the additive term. This model predicted the cross line means adequately – model fit could not be improved by adding dominance and epistasis (Table 2). Sex-specific analyses (not shown) produced very similar results.

Discussion

One main finding emerging from our analysis of lake–stream stickleback line crosses is that the differences in foraging-related traits observed between the ecotypes in the Misty watershed have a strong genetic basis, as opposed to being obviously phenotypically plastic. This finding is consistent with previous analyses comparing field-caught and laboratory-reared stickleback from the same watershed (Lavin & McPhail, 1993; Sharpe *et al.*, 2008; Hendry *et al.*, 2011). Divergence in morphology therefore has the potential to contribute to reproductive isolation not only through selection against migrants between habitats, but potentially also through selection against first- and later-generation hybrids arising from interbreeding between the ecotypes.

Another main finding is striking nonadditive effects in the inheritance of body shape traits. The analysis of RW1, a multivariate compound variable capturing primarily variation in overall body depth and snout length, indicated strong sexual dimorphism, and dominance and epistasis differing in magnitude between the sexes. This finding disagrees with the additive inheritance observed

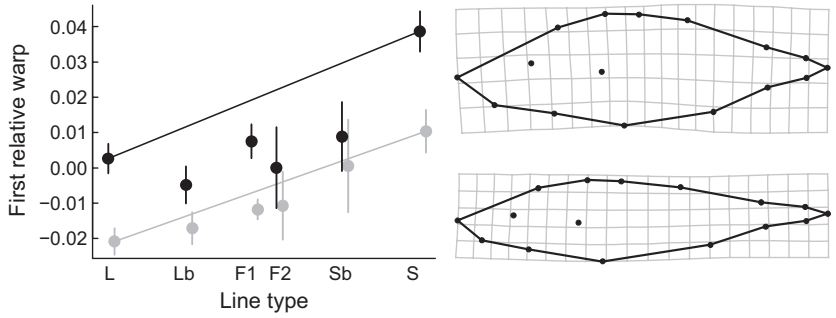


Fig. 1 Body shape (first relative warp, RW1) of pure lake (L) and stream (S) stickleback ecotypes and their F1 and F2 hybrids (F1 and F2) and backcrosses (Lb, Sb). Shown are line means with 95% confidence intervals, based on shape analysis using family consensus configurations (for sample sizes, see Table 1). Data for males and females are shown in black and grey. The lines connecting the lake and stream means in each sex indicate the mean for each cross type expected under purely additive inheritance. The deformation grids visualize the lowest and highest observed line means (stream male at top and lake female at bottom). Note the dominance of lake-derived genetic factors, especially in males.

Table 2 Hierarchical comparison of genetic models fitted to geometric morphometric body shape (first relative warp, RW1), univariate body depth, and gill raker number for lake and stream stickleback lines and their derivatives. For RW1 and body depth, we present results from analyses combining male and female data. For gill raker number, only the analysis with the sexes pooled is presented because no sex-related effects were present. Models with greatest explanatory power, as indicated by second-order information criteria (AIC_c), are given in bold (for details, see text). The number of model parameters, including the intercept and residual variance, are given in parentheses.

Model	AIC_c
RW1	
s + [a]	−720.7 (4)
s + [a] + [d]	−747.4 (5)
s + [a] + [d] + s*[d]	−750.2 (6)
s + [a] + [d] + s*[d] + [aa] + [ad] + [dd]	−759.2 (9)
Body depth	
s + [a]	205.7 (4)
s + [a] + [d]	190.9 (5)
s + [a] + [d] + s*[d]	192.9 (6)
s + [a] + [d] + [aa] + [ad] + [dd]	159.0 (8)
Gill raker number	
[a]	92.0 (3)
[a] + [d]	93.2 (4)
[a] + [d] + [aa] + [ad] + [dd]	94.3 (7)

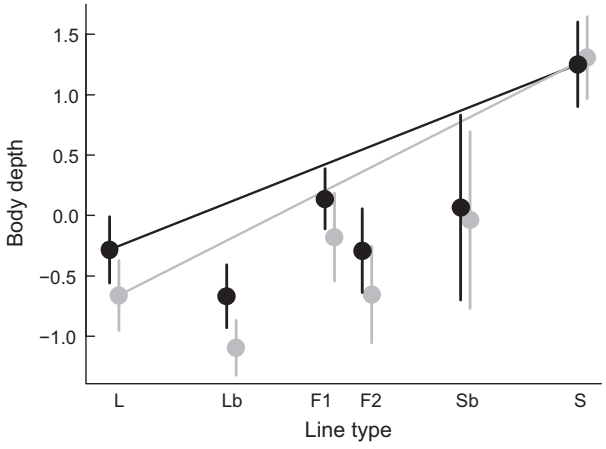


Fig. 2 Size-corrected body depth (measured as linear inter-landmark distance) in lake and stream stickleback ecotypes and their line cross derivatives. Shown are means with 95% confidence intervals for males (black) and females (grey), calculated using family averages as data points (for sample sizes, see Table 1). Note that both hybrids and backcrosses resemble the lake phenotype and hence deviate from the additive expectation (lines connecting the pure ecotype means).

for the divergence in a compound shape trait between marine and stream stickleback (Schluter *et al.*, 2004).

The analysis of the univariate body-shape traits (body depth and snout length), however, raises uncertainties about some interpretations based solely on RW1.

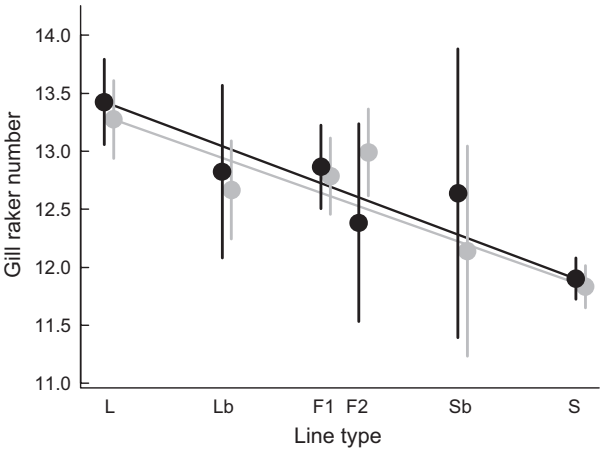


Fig. 3 Gill raker number in lake and stream stickleback ecotypes and their line cross derivatives. Shown are means with 95% confidence intervals for males (black) and females (grey), calculated using family averages as data points (for sample sizes, see Table 1). Line means are well predicted by additive gene action (lines connecting the pure ecotype means).

Although strong dominance and epistatic effects were also present for univariate body depth, all sex-related differences in genetic architecture were much weaker. Snout length, by contrast, exhibited striking sexual dimorphism [as also reported by Leinonen *et al.* (2011) for European populations] but no appreciable differentiation between cross lines (Fig. A1). In the light of a recent simulation study (Berner, 2011), the discrepancy between the geometric morphometric and the univariate shape trait analyses strongly suggests that RW1 is an artefact. That study demonstrated that principal component analysis systematically introduces artificial covariance among the constituent variables because principal components are constrained to be orthogonal to each other (for further details, see Berner, 2011). Most likely, RW1 in our study thus confounds genetically independent axes of variation (i.e. sexual dimorphism and habitat-related divergence) due to the orthogonalization of the weight matrix. In line with this view, a mapping study failed to identify reliable QTLs for stickleback body shape quantified as RW scores, whereas such QTLs emerged when mapping raw landmark coordinates (Albert *et al.*, 2008). (Presumably QTLs would also emerge when analysing univariate body depth measurements, but this has yet to be performed.) Together, these results suggest that although shape analysis based on relative warps facilitates pattern recognition, these compound traits are inappropriate phenotypes for genetic investigation. The finding of strong dominance and epistasis in body depth, however, is robust to the method of analysis and adds to an increasing number of examples for nonadditive inheritance of phenotypic population differentiation (reviewed in Roff & Emerson, 2006).

A third main finding is simple inheritance for gill raker number: sexual dimorphism was absent, and the line cross means were adequately predicted by an additive model. Both findings are consistent with a line cross analysis using sympatric lacustrine stickleback species (Hatfield, 1997). The difference in inheritance between body depth and gill raker number has implications for reproductive isolation between the stickleback ecotypes found in the contiguous lake and stream habitats in the Misty system. Given divergent selection on both phenotypes between the habitats, we predict that gill raker number should contribute *symmetrically* to reproductive isolation. The reason is that phenotypes of hybrids in the first few generations at least will lie between the pure ecotypes and should therefore be selected against in both environments (Schluter, 2000; Rundle & Nosil, 2005). By contrast, we predict that divergent selection on body depth will contribute *asymmetrically* to reproductive isolation. Because the genetic factors underlying shallow bodies in lake fish display strong dominance on average, many hybrids will resemble the lake phenotype even beyond the first generation. Hybrid body depth should therefore be appropriate for the lake habitat, potentially contributing to gene flow from the stream to the lake population, particularly for genes related to body depth. In streams, however, hybrid performance should be substantially reduced, restricting gene flow from the lake population, again especially at genes related to body depth.

The aforesaid mechanisms may well operate in nature because a narrow zone is known where Misty Lake and inlet stream ecotypes do indeed hybridize (J.S. Moore, E.B. Taylor, and A.P. Hendry unpublished). The existence of this hybrid zone also opens up the opportunity for evaluating two conditions regarding our predictions of how morphological divergence could influence reproductive isolation. First, the genetic architecture of divergence estimated under laboratory conditions needs to parallel the genetic architecture expressed in nature. This should ideally be confirmed through further work, given that several line cross analyses in other systems indicate nontrivial genotype–environment interactions (Armbruster *et al.*, 1997; Fritz *et al.*, 2006; Demuth & Wade, 2007; Fuller, 2008). An approach that could be taken is to sample admixed individuals from the hybrid zone and to relate their phenotype to their hybrid status estimated by using neutral markers. Second, a reasonably strong link needs to exist between the morphological traits and overall fitness. Such a link is strongly indicated by the *repeated* lake–stream divergence in body depth and gill raker number in numerous independent watersheds (see references mentioned earlier). Of course, many other traits likely contribute to adaptive lake–stream divergence, and so formal analyses would be valuable. For example, a direct quantification of the trait-fitness association could be achieved by estimating selection gradients on the traits of admixed individuals in both habitats (Lexer *et al.*, 2003). Admixed individuals may further be used for the mapping of QTLs underlying the additive and nonadditive genetic effects observed in the present study (Buerkle & Lexer, 2008).

To summarize, we find striking differences in additive and nonadditive genetic contributions to lake–stream divergence between two key morphological traits in stickleback. We therefore suggest that each trait's contribution to maintaining the genetic integrity of the ecotypes in parapatry will differ qualitatively. It would now be valuable to perform similar analyses in other lake–stream systems to see whether our results are general. Furthermore, a better understanding of the interplay between genetic architecture, adaptive divergence, and reproductive isolation could be gained through studies that link trait values and performance in both environments, and hence quantify the fitness consequences of hybridization stemming from each trait. Finally, the genetic architecture of divergence in foraging morphology should now be investigated from a molecular angle.

Acknowledgments

The generation and maintenance of the stickleback lines was greatly aided through the hard work of M. Boisjoly, E. Brander, S. Clemmensen, L. Delaire, M. Delcourt, N. Earl, C. Fuentes-Ortega, K. Hudson, S. Kalbfleisch, C. Macnaughton, N. Majorkiewicz, J.-S. Moore, H. Roffey, K. Tombak, and the personnel of the McGill Phytotron. Erik Postma provided input on the analysis at an early stage. Field and laboratory work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to APH. DB and ACG were supported financially by the Swiss National Science Foundation (grants: PBBSA-111216 and Ambizione PZ00P3_126391/1), the Janggen-Pöhn Foundation, the Roche Research Foundation, the Stiefel-Zangger Foundation, and the Research Fund of the University of Basel. RK was supported by an FQRNT postdoctoral fellowship. JAMR was supported by the Research Foundation – Flanders (FWO-Vlaanderen) and the K.U. Leuven (project GOA/2006/06). KR was supported by the Swedish Research council (VR).

References

Aguirre, W.E. 2009. Microgeographical diversification of threespine stickleback: body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. *Biol. J. Linn. Soc.* **98**: 139–151.

Albert, A.Y.K., Sawaya, S., Vines, T.H., Knecht, A.K., Miller, C.T., Summers, B.R. *et al.* 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. *Evolution* **62**: 76–85.

Armbruster, P., Bradshaw, W.E. & Holzapfel, C.M. 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution* **51**: 451–458.

Arnold, M.L. 1997. *Natural Hybridization and Evolution*. Oxford University, New York.

Arnold, M.L. & Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents? *Trends Ecol. Evol.* **10**: 67–71.

Barson, N.J., Knight, M.E. & Turner, G.F. 2007. The genetic architecture of male colour differences between a sympatric Lake Malawi cichlid species pair. *J. Evol. Biol.* **20**: 45–53.

Barton, N.H. 2001. The role of hybridization in evolution. *Mol. Ecol.* **10**: 551–568.

Barton, N.H. & Hewitt, G.M. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* **16**: 113–148.

Berner, D. 2011. Size correction in biology: how reliable are approaches based on (common) principal component analysis? *Oecologia*, in press.

Berner, D., Adams, D.C., Grandchamp, A.-C. & Hendry, A.P. 2008. Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. *J. Evol. Biol.* **21**: 1653–1665.

Berner, D., Grandchamp, A.-C. & Hendry, A.P. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. *Evolution* **63**: 1740–1753.

Berner, D., Roesti, M., Hendry, A.P. & Salzburger, W. 2010. Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Mol. Ecol.* **19**: 4963–4978.

Blake, R.W. 2004. Fish functional design and swimming performance. *J. Fish Biol.* **65**: 1193–1222.

Buerkle, C.A. & Lexer, C. 2008. Admixture as the basis for genetic mapping. *Trends Ecol. Evol.* **23**: 686–694.

Burnham, K.P. & Anderson, D.R. 2002. *Model Selection and Multimodel Inference: A Pratical Information-Theoretic Approach*, 2nd edn. Springer, New York.

Coyne, J.A. & Orr, H.A. 2004. *Speciation*. Sinauer Associates, Sunderland.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *J. Evol. Biol.* **21**: 1460–1469.

Czesak, M.E., Knee, M.J., Gale, R.G., Bodach, S.D. & Fritz, R.S. 2004. Genetic architecture of resistance to aphids and mites in a willow hybrid system. *Heredity* **93**: 619–626.

Delcourt, M., Räsänen, K. & Hendry, A.P. 2008. Genetic and plastic components of divergent male intersexual behavior in Misty lake/stream stickleback. *Behav. Ecol.* **19**: 1217–1224.

Demuth, J.P. & Wade, M.J. 2007. Population differentiation in the beetle *Tribolium castaneum*. I. Genetic architecture. *Evolution* **61**: 494–509.

Dobzhansky, T. 1951. *Genetics and the Origin of Species*, 3rd edn. Columbia University, New York.

Fritz, R.S., Hochwender, C.G., Albrectsen, B.R. & Czesak, M.E. 2006. Fitness and genetic architecture of parent and hybrid willows in common gardens. *Evolution* **60**: 1215–1227.

Fuller, R.C. 2008. Genetic incompatibilities in killifish and the role of environment. *Evolution* **62**: 3056–3068.

Gross, H.P. & Anderson, J.M. 1984. Geographic variation in the gillrakers and diet of European sticklebacks, *Gasterosteus aculeatus*. *Copeia* **1**: 87–97.

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

Hatfield, T. 1997. Genetic divergence in adaptive characters between sympatric species of stickleback. *Am. Nat.* **149**: 1009–1029.

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

Hendry, A.P. & Taylor, E.B. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs *Evolution* **58**: 2319–2331.

Hendry, A.P., Taylor, E.B. & McPhail, J.D. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. *Evolution* **56**: 1199–1216.

Hendry, A.P., Hudson, K., Walker, J.A., Räsänen, K. & Chapman, L.J. 2011. Genetic divergence in morphology-performance mapping between Misty Lake and inlet stickleback. *J. Evol. Biol.* **24**: 23–35.

Lavin, P.A. & McPhail, J.D. 1993. Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? *Can. J. Zool.* **71**: 11–17.

Leinonen, T., Cano, J.M. & Merila, J. 2011. Genetic basis of sexual dimorphism in the threespine stickleback *Gasterosteus aculeatus*. *Heredity* **106**: 218–227.

Lexer, C., Randell, R.A. & Rieseberg, L.H. 2003. Experimental hybridization as a tool for studying selection in the wild. *Ecology* **84**: 1688–1699.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland.

Mather, K. & Jinks, J.L. 1982. *Biometrical Genetics – The Study of Continuous Variation*. Chapman & Hall, London.

Moore, J.S. & Hendry, A.P. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. *Evol. Ecol. Res.* **7**: 1–16.

R Development Core Team 2010. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rego, C., Santos, M. & Matos, M. 2007. Quantitative genetics of speciation: additive and non-additive genetic differentiation between *Drosophila madeirensis* and *Drosophila subobscura*. *Genetica* **131**: 167–174.

Reimchen, T.E., Stinson, E.M. & Nelson, J.S. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Can. J. Zool.* **63**: 2944–2951.

Reist, J.D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Can. J. Zool.* **63**: 1429–1439.

Reusch, T.B.H., Wegner, K.M. & Kalbe, M. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. *Mol. Ecol.* **10**: 2435–2445.

Rieseberg, L.H. 1995. The role of hybridization in evolution: old wine in new skins. *Am. J. Bot.* **82**: 944–953.

Robinson, B.W. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour* **137**: 865–888.

Roff, D.A. & Emerson, K. 2006. Epistasis and dominance: evidence for differential effects in life-history versus morphological traits. *Evolution* **60**: 1981–1990.

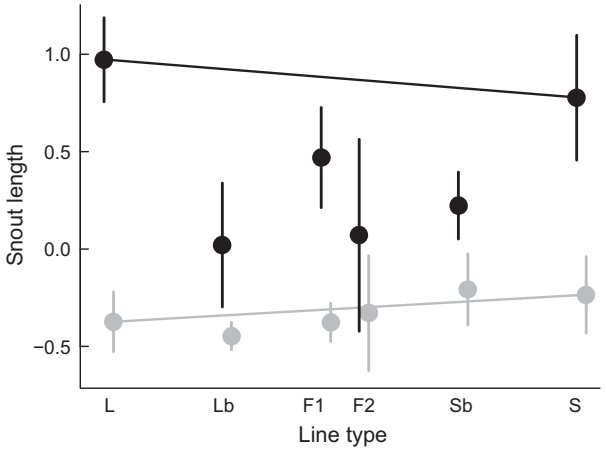
Rohlf, F.J. 2001. *tpsDig, tpsRelw, tpsRegr*. Distributed by the author. Department of Ecology and Evolution, State University of New York, Stony Brook, NY.

Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecol. Lett.* **8**: 336–352.

Schluter, D. 1993. Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology* **74**: 699–709.
Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University, Oxford.
Schluter, D., Clifford, E.A., Nemethy, M. & McKinnon, J.S. 2004. Parallel evolution and inheritance of quantitative traits. *Am. Nat.* **163**: 809–822.
Sharpe, D.M.T., Räsänen, K., Berner, D. & Hendry, A.P. 2008. Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation. *Evol. Ecol. Res.* **10**: 849–866.
Sobel, J.M., Chen, G.F., Watt, L.R. & Schemske, D.W. 2010. The biology of speciation. *Evolution* **64**: 295–315.
Thibert-Plante, X. & Hendry, A.P. 2011. The consequences of phenotypic plasticity for ecological speciation. *J. Evol. Biol.* **24**: 326–342.
Thompson, C.E., Taylor, E.B. & McPhail, J.D. 1997. Parallel evolution of lake-stream pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA variation. *Evolution* **51**: 1955–1965.
Walker, J.A. 1997. Ecological morphology of lacustrine three-spine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* **61**: 3–50.
Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *Am. Zool.* **24**: 107–120.

Appendix

Figure A1 Size-corrected snout length (measured as linear inter-landmark distance) in lake (L) and stream (S) stickleback ecotypes and their line cross derivatives. Shown are means with 95% confidence intervals for males (black) and females (grey), calculated using family averages as data points (for sample sizes, see Table 1). Note the striking sexual dimorphism (especially in the pure lines) and the absence of ecotype differences.



Data deposited at Dryad: doi: 10.5061/dryad.6vq04

Received 11 February 2011; revised 3 May 2011; accepted 16 May 2011