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Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation

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ABSTRACT

Question: Do the morphological differences between wild lake and stream stickleback have a genetic basis?

Organisms: Wild-caught and laboratory-reared threespine stickleback (*Gasterosteus aculeatus*) from Misty Lake, and from its inlet and outlet streams.

Methods: A common-garden experiment was used to examine the genetic and environmental components of morphological variation. Morphology was quantified through multivariate body shape (geometric morphometrics) and through a suite of linear measurements.

Conclusions: The most striking morphological differences between the inlet and lake populations have a genetic basis, whereas those between the outlet and lake populations do not. Most notably, inlet fish have genetically deeper bodies, shorter pelvic and dorsal spines, and deeper caudal peduncles than do lake or outlet fish. All traits showed substantial plasticity; however, the relative differences between ecotypes were similar in the wild and the laboratory. These genetically based morphological differences may contribute to several ecologically dependent reproductive barriers between lake and inlet stickleback.

Keywords: adaptive divergence, common-garden experiment, geometric morphometrics, phenotypic change vector analysis, phenotypic plasticity.

INTRODUCTION

Phenotypic variation among natural populations sometimes reflects genetic adaptation to local selective pressures (e.g. Endler, 1986; Schluter, 2000). At other times, it reflects plastic responses to local environmental conditions (e.g. Levins, 1969; Berven *et al.*, 1979; James, 1983). Most of the time, however, it probably reflects a combination of both genetic and plastic influences, which

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may interact in a variety of complicated ways (Conover and Schultz, 1995; Schlichting and Pigliucci, 1998; West-Eberhard, 2003). Disentangling the relative contributions of genetic and environmental effects is particularly important for understanding the factors that promote or constrain evolutionary diversification. We undertake this task for a natural system that we have been using to study adaptive divergence and its contributions to reproductive isolation – lake and stream populations of threespine stickleback (*Gasterosteus aculeatus*) in Misty Lake, British Columbia.

Interactions between natural selection and gene flow are often invoked to explain patterns of phenotypic variation in the wild; however, such interpretations require a good understanding of genetic and plastic contributions to phenotypic variation (Crispo, 2008). In the Misty system, for example, some lake and stream stickleback differ phenotypically in ways that are thought to reflect the action of divergent natural selection. In other lake–stream pairs, phenotypic divergence is quite low, and we have argued that high gene flow is the reason (Hendry *et al.*, 2002; Hendry and Taylor, 2004; Moore and Hendry, 2005; Moore *et al.*, 2007; Berner *et al.*, 2008; Delcourt *et al.*, in press). Both of these inferences (that selection promotes divergence and that gene flow constrains it) implicitly assume that the observed phenotypic divergence has a genetic basis. It is also possible, however, that phenotypic differences observed in the wild reflect plastic responses to different environments (rather than adaptive divergence in response to natural selection), whereas phenotypic similarities reflect plastic convergence *in spite of* genetic differences (rather than the constraining influence of gene flow). Gene flow may even be facilitated by plasticity (see below), which may then feed back to favour the evolution of increased plasticity rather than strong genetic divergence (Via and Lande, 1985; Scheiner, 1998; Sultan and Spencer, 2002; Crispo, 2008).

Further to the above, genetic and plastic contributions to trait variation will also influence any ecologically dependent reproductive isolation. One factor contributing to such isolation in the Misty system might be selection against migrants between the lake and streams (Hendry *et al.*, 2002). The strength of this barrier, however, would be sensitive to the genetic versus plastic basis for the phenotypic variation. If adaptive phenotypic differences have a strong genetic basis, or reflect permanent plastic developmental responses to the home environment, then individuals migrating from one environment to another should be maladapted and thus suffer reduced fitness (Hendry, 2004; Nosil *et al.*, 2005). Similarly, hybrid offspring may fall between the ecological niches of their parents, and thus be selected against (Schluter, 2000; Coyne and Orr, 2004; Rundle and Nosil, 2005). If, on the other hand, phenotypic variation in the wild is the result of plasticity, then individuals moving from one environment to another (or any resulting hybrids) may be able to express the phenotype that is adaptive in the new environment – and thereby suffer little negative fitness consequence. This outcome will, of course, depend on (1) the magnitude and direction of plasticity in relation to the local optimum, (2) the lability of the traits at the developmental stage when individuals switch environments, and (3) the costs of expressing plasticity. Depending on these various factors, plasticity may then increase or decrease the strength of selection on the same and other traits (Price *et al.*, 2003; Ghalambor *et al.*, 2007; Crispo, 2008).

The above considerations highlight that reliable inferences regarding the factors promoting or constraining diversification require information on the genetic and plastic basis for apparently adaptive phenotypic traits (Crispo, 2008). Previous work on lake and stream stickleback has shown that at least some of the phenotypic differences between ecotypes are maintained in a common-garden laboratory environment (Lavin and McPhail, 1993; Hendry *et al.*, 2002), suggesting a genetic basis to these traits. These earlier studies, however, assessed only a

few traits and one of the most important traits (body depth) was evaluated for only one type of linear measurement. We therefore undertook a more comprehensive study of morphological variation among wild and laboratory-reared lake and stream stickleback from the Misty Lake system. In the wild, one of the stream populations (Misty Inlet) shows dramatic phenotypic divergence from the lake, whereas the other (Misty Outlet) does not (Hendry *et al.*, 2002; Moore *et al.*, 2007). If these patterns have a genetic basis (as previously assumed), then they should be maintained in a common-garden rearing environment. The extent to which the traits are determined by genetic versus plastic responses will have important implications for the interplay between adaptive divergence, gene flow, and ecological reproductive isolation in this system.

METHODS

Wild-caught fish

Wild fish were caught using unbaited minnow traps at several of the sites described by Moore and Hendry (2005). In 2003, we sampled Misty Inlet site 3 ($n = 24$), Misty Outlet site 4 ($n = 17$), and Misty Lake site 1 ($n = 17$). In 2004, we sampled Misty Inlet site 3 ($n = 21$), Misty Inlet site 4 ($n = 11$), Misty Outlet site 4 ($n = 16$), and Misty Lake site 1 ($n = 23$). Sample sizes were not larger because the lake and inlet populations are of conservation concern (COSEWIC, 2006). The collected fish were killed with an overdose of tricaine methanesulphonate (MS-222) and immediately photographed with a digital camera. For the photographs, each fish was placed left side up in a natural position on a standard grid-ruled (1-mm) background. When necessary, small pins were used to extend the spines and fins (see Fig. 1). Afterwards, each fish was dissected to determine its sex.

Common-garden experiment

In June 2004, we collected mature stickleback from Misty Lake (site 1), Misty Inlet (site 4), and Misty Outlet (site 4). Using standard artificial crossing methods (Hatfield and Schluter, 1996), we generated eight full sibling families for the lake, seven for the outlet, and four for the inlet (at the time of sampling few gravid females were available in the inlet). The fertilized eggs were shipped back to McGill University, where they hatched and were then reared until maturity using standard protocols (Delcourt *et al.*, in press). The fish from a given family were maintained at approximately equal densities (25 fish per 100 litres) in multiple aquaria, and different families were not mixed. Each family was fed an identical diet that varied with developmental stage from live brine shrimp nauplii (*Artemia sp*), frozen brine shrimp, and live blackworms (*Lumbriculus* sp.) to frozen blood worms (Chironomid larvae).

The fish were maintained under 'summer' conditions (17°C, 16 h light and 8 h dark) until March 2005, when they were switched to 'winter' conditions (12°C, 8 h light and 16 h dark). Different tanks were then switched back to the above 'summer' conditions at different times in September/October 2005, so as to stagger their availability for other experiments (Delcourt *et al.*, in press). When each fish matured (October–December 2005), it was individually photographed as described above for the wild fish. For the analyses that follow, we randomly selected three mature males and three mature females from each of four families of each ecotype, thus equalizing the contribution of sex and family to the final data set ($n = 72$).

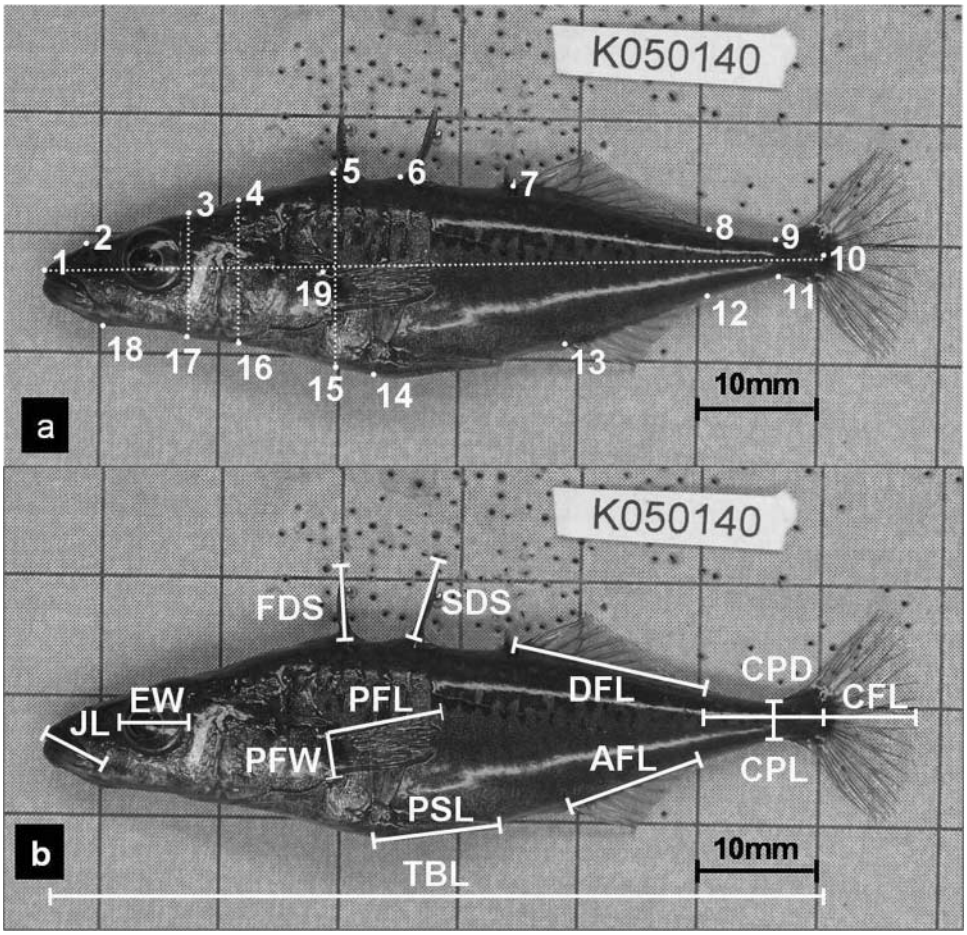

Fig. 1. Depiction of the morphological traits used for analysis. Panel (a) shows the landmarks and semi-landmarks used in geometric morphometric analyses. The 14 landmarks are: anterior tip of upper jaw (1), tip of nasal bone (2), anterior insertion of first dorsal spine (5), anterior insertion of second dorsal spine (6), anterior insertion of first dorsal fin ray (7), insertion of dorsal fin membrane on dorsal midline (8), origin of caudal fin membrane on dorsal midline (9), caudal border of hypural plate at lateral midline (10), origin of caudal fin membrane on ventral midline (11), insertion of anal fin membrane on ventral midline (12), anterior insertion of first anal fin ray (13), anterior insertion of pelvic spine (14), posterior edge of angular (18), and anterior insertion of first pectoral fin ray (19). The five semi-landmarks are: intersection of dorsal outline with posterior edge of eye orbit (3), intersection of dorsal outline with grid line (4), intersection of ventral outline with anterior insertion of first dorsal spine (15), intersection of ventral outline with grid line (16), and intersection of ventral outline with posterior edge of eye orbit (17). Panel (b) shows the linear trait measurements: total body length (TBL), jaw length (JL), eye width (EW), pectoral fin width (PFW), pectoral fin length (PFL), pelvic spine length (PSL), dorsal fin length (DFL), anal fin length (AFL), caudal peduncle depth (CPD), caudal peduncle length (CPL), caudal fin length (CFL), first dorsal spine length (FDS), and second dorsal spine length (SDS).

Measuring morphology

All photographs were processed in random order by a single individual (D.M.T.S.) who was blind to each fish's capture site. TPSDig software (v. 2.05, © Rohlf 2006) was used to record the X,Y coordinates of 19 points on each specimen (Fig. 1a). Fourteen of these points were discrete homologous landmarks, based mostly on Walker (1997), whereas the remaining five points were 'semi-landmarks' intended to measure important shape variation that is not captured by true landmarks (Zelditch *et al.*, 2004). Semi-landmarks were positioned by reference to a grid superimposed onto the image of each fish. This grid consisted of one line along the lateral axis of the fish (tip of the upper jaw to the end of the hypural plate) and three lines perpendicular to this lateral axis. The first of these perpendicular lines was positioned at the posterior edge of the eye orbit, the last line at the anterior insertion of the first dorsal spine, and the middle line at one-third of the lateral distance from the first to the last line. Semi-landmarks were then placed where these three perpendicular lines intersected the outline of the fish (Fig. 1a).

TPSRelw (v. 1.42, © Rohlf 2005) was used to calculate the centroid size of each fish as the square root of the sum of the squared distances from each landmark and semi-landmark to the centroid of the landmark configuration. Each fish's configuration of landmarks was then scaled to unit centroid size, and superimposed onto the mean, or 'consensus', configuration. This procedure uses generalized least squares Procrustes superimposition to minimize the sum of squared distances between homologous landmarks (Rohlf and Slice, 1990). Deviations of each fish from the consensus configuration were then expressed in terms of affine (uniform) and non-affine (partial warp) components (Zelditch *et al.*, 2004). Finally, we extracted 'relative warps' (principal components) from an analysis that included all of the affine and non-affine components.

In addition to digitizing (semi-)landmarks, TPSDig was used for linear measurements of specific traits related to swimming (fin lengths and the caudal peduncle depth and length), defence (spine lengths), and foraging (eye width and jaw size) (see Fig. 1b for details). Jaw length and pelvic spine length are known to differ between lake and stream stickleback (Lavin and McPhail, 1993; Hendry *et al.*, 2002; Hendry and Taylor, 2004), but the other traits have not previously been investigated.

Statistical analysis – potential complicating factors

All statistical analyses were performed in JMP (v. 5.1.2, SAS Institute), unless otherwise noted. We began by considering three sources of variation that might complicate comparisons among populations and between rearing environments. First, we investigated the precision of our measurements by repeating all of the above procedures on 15 randomly selected photographs. Measurement error was always very low (correlation between repeated estimates of relative warps (RWs) and linear measurements: $r = 0.836–0.997$).

Second, we investigated the magnitude of inter-annual variation for the wild-caught samples. For this, we used two MANCOVAs, one for body shape variables (uniform components and partial warps) and one for linear measurements. Predictor variables in each case were: year, ecotype (lake, inlet or outlet), sex, and body size (centroid size for the shape analysis and log10 body length for the linear trait analysis). Variation between years was significant for both body shape ($F_{34,87} = 10.83$, $P < 0.001$) and linear measurements ($F_{12,105} = 9.33$, $P < 0.001$). Part of this variation may be biologically significant, but part also

arose from differences in the way the fish were photographed in different years. For this reason, we performed all of the following analyses twice: once with the 2003 wild-caught samples and once with the 2004 wild-caught samples. Conclusions were the same in either case, and so we here report only the results from analyses that used the 2004 samples. This decision was made because the parents of the laboratory-reared fish came from the specific sites sampled in 2004.

Third, we evaluated the effects of full-sib family structure in the laboratory-reared fish to account for the non-independence of individuals originating from the same families. Here we tested for an ecotype effect in models that included family nested within ecotype, as well as appropriate covariates (centroid size for geometric morphometrics and log10 body length for the linear measurements). The effect of ecotype was significant, or approached significance, in most analyses ($F_{68,48} = 3.57$, $P < 0.001$ in MANCOVA including the uniform components and all partial warps; $F_{24,96} = 1.50$, $P = 0.086$ in MANCOVA including all the log10 linear measurements; $F_{2,9} = 3.88$, $P = 0.026$ in ANCOVA on RW1; $F_{2,9} = 13.75$, $P < 0.001$ in ANCOVA on RW2). Accounting for family-level variation thus did not eliminate the main differences between the ecotypes. For this reason, and because family structure was not known for the wild fish, all subsequent analyses are presented without family being included as a term in the model.

Statistical analysis – ecotypes and rearing environments

We examined variation among ecotypes (lake, inlet, outlet) and between rearing environments (wild-caught vs. laboratory-reared) through the use of two MANCOVAs, one for body shape and one for the linear measurements. For body shape, response variables were the two uniform components and the 16 partial warps. For linear measurements, response variables were all 12 individual measurements (log10 transformed). Predictor variables in both MANCOVAs were ecotype, rearing environment, sex, body size (centroid size for the shape analysis and log10 body length for the linear trait analysis), and all possible interactions. We then sequentially removed all non-significant predictor variables to settle on a 'reduced' model for interpretation. For each factor remaining in a model, MANCOVA was used to generate canonical variates (CVs) that optimally explained variation associated with that factor. For geometic morphometrics, this variation was visualized by using the CVs to generate thin plate spline transformations of landmark configurations in TPSRegr (v. 1.31, © Rohlf 2005).

We supplemented the above MANCOVAs with univariate analyses of the most important relative warps. These additional analyses were valuable because (1) RWs are constructed without reference to *a priori* defined factors of interest (in contrast to the CVs), (2) RWs often isolate specific major components of shape variation, and (3) individual RWs can be used in *post hoc* comparisons among specific groups. For each RW of interest (see below), we used ANCOVA to test for effects of ecotype, rearing environment, sex, centroid size, and all possible interactions. As above, all non-significant predictor variables were sequentially removed from the final model. *Post hoc* Tukey tests were used to compare least square means between specific groups.

Finally, we used phenotypic change vector analysis [PCVA (Collyer and Adams, 2007)] to examine the details of multivariate divergence in body shape and linear measurements. We specifically asked how the multivariate vector connecting ecotypes might differ between rearing environments in magnitude (difference in vector length) and orientation (angle

between the vectors). The first step was to use the above MANCOVAs to generate multivariate least-square (LS) means for every ecotype × habitat combination (only significant terms used). The next step was to express the PCVs as vectors connecting the LS means for each of the three pairwise ecotype combinations (lake–inlet, lake–outlet, and inlet–outlet). This was done separately for the wild-caught and laboratory-reared fish. For each ecotype combination, we then calculated the difference in magnitude (vector length) between the two environment-specific PCVs, as well as the difference in their orientation (angle between the vectors). The final step was to use residual randomization to create a random distribution for the difference in magnitude and orientation, against which the observed values could be evaluated. [For further details on the procedure, see Collyer and Adams (2007).] PCVA was carried out in R (R Development Core Team, 2006). For the linear measurements, PCVA was visualized by plotting ecotype × environment LS means obtained from the above MANCOVA models along their first two principal components. Standard errors around the LS means were calculated after projecting individuals on these PC axes.

RESULTS

Relative warps

RW1 captured 29.4% of the total variation in body shape among all individuals and was related to changes in fin position and abdomen distension (Fig. 2a). Individuals with increasingly positive scores showed a constriction of the abdomen, a more posterior insertion of the pectoral fin, and lengthening of the dorsal and anal fins (by a displacement of the posterior insertion of the fins towards the caudal region). RW2 explained 20.4% of the shape variation and was related to body depth along the entire longitudinal axis of the fish (Fig. 2b). Individuals with increasingly positive scores showed a narrowing of the body along the entire length of the fish, and a slight anterior displacement of the pectoral fin. RW3 and RW4 were not considered further because they were related to bending associated with subtle variation in how a fish was positioned for the photographs.

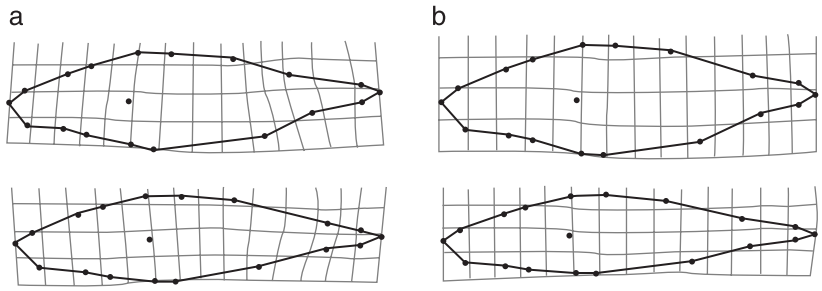

Fig. 2. Thin plate spline deformations showing the observed extremes in the entire data set for RW1 (a) and RW2 (b). In each case, variation is depicted from the lowest observed score (top panel) to the highest observed score (bottom panel). See text for further details.

Genetic and environmental influences on body shape

MANCOVA revealed that variation in stickleback body shape had a strong genetic (ecotype) component (Table 1). The most striking pattern was that inlet fish were deeper bodied along most of their length than lake and outlet fish, a pattern clear both in the wild and in the laboratory (CV_E; Fig. 3). ANCOVA of RW2, which captured similar variation, closely confirmed this result (Fig. 4, Table 2). Variation along RW1 was more difficult to interpret, given that the differences among ecotypes were generally not statistically

Table 1. Results of MANCOVA for variation in body shape among Misty Lake stickleback

Source of variation	*F*	d.f.	*P*	Partial variance explained (%)
Ecotype (E)	4.89	68, 194	<0.001	63.2
Rearing environment (RE)	3.27	34, 97	<0.001	53.4
RE × E	1.38	68, 194	0.046	32.6
Centroid size (CS)	3.14	34, 97	<0.001	52.4
E × CS	1.54	68, 194	0.012	35.1
RE × CS	2.97	34, 97	<0.001	51.0
RE × E × CS	1.53	68, 194	0.013	34.8

Note: 'Ecotypes' are lake, inlet, and outlet. 'Rearing environments' are the wild and the laboratory. *F*-values were approximated using Wilk's λ for each term. Partial variance explained by each effect was calculated using Wilk's partial η^2 after Langerhans and DeWitt (2004).

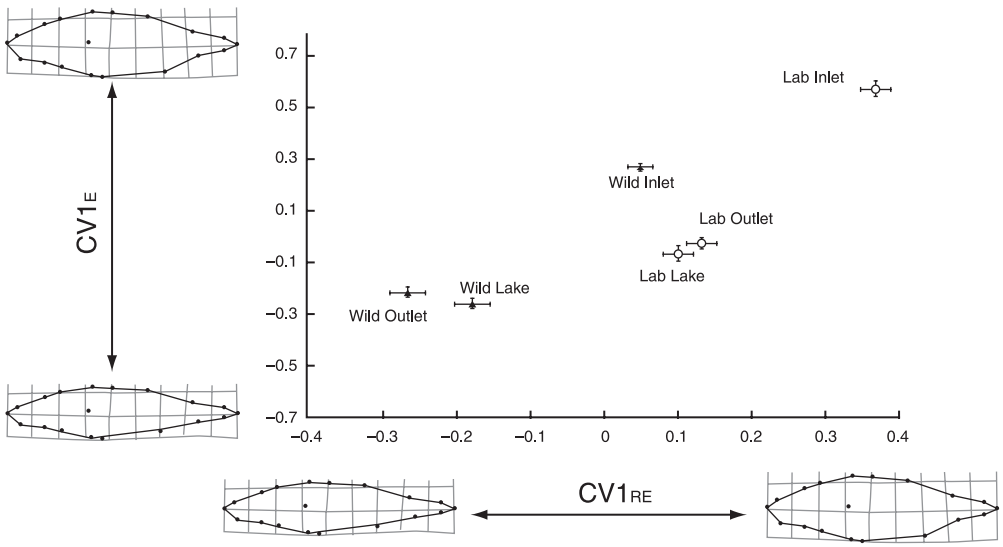

Fig. 3. Variations in body shape among Misty Lake stickleback attributable to ecotype ($CV1_E$) and rearing environment ($CV1_{RE}$). Symbols represent mean scores (± standard errors) for each population along each CV. Thin plate spline deformations show the landmark configuration that corresponds to the maximum or minimum observed scores for each CV. Note that CV axes are constructed to maximize the phenotypic divergence between groups for a given factor, and do not necessarily represent true phenotypic space.

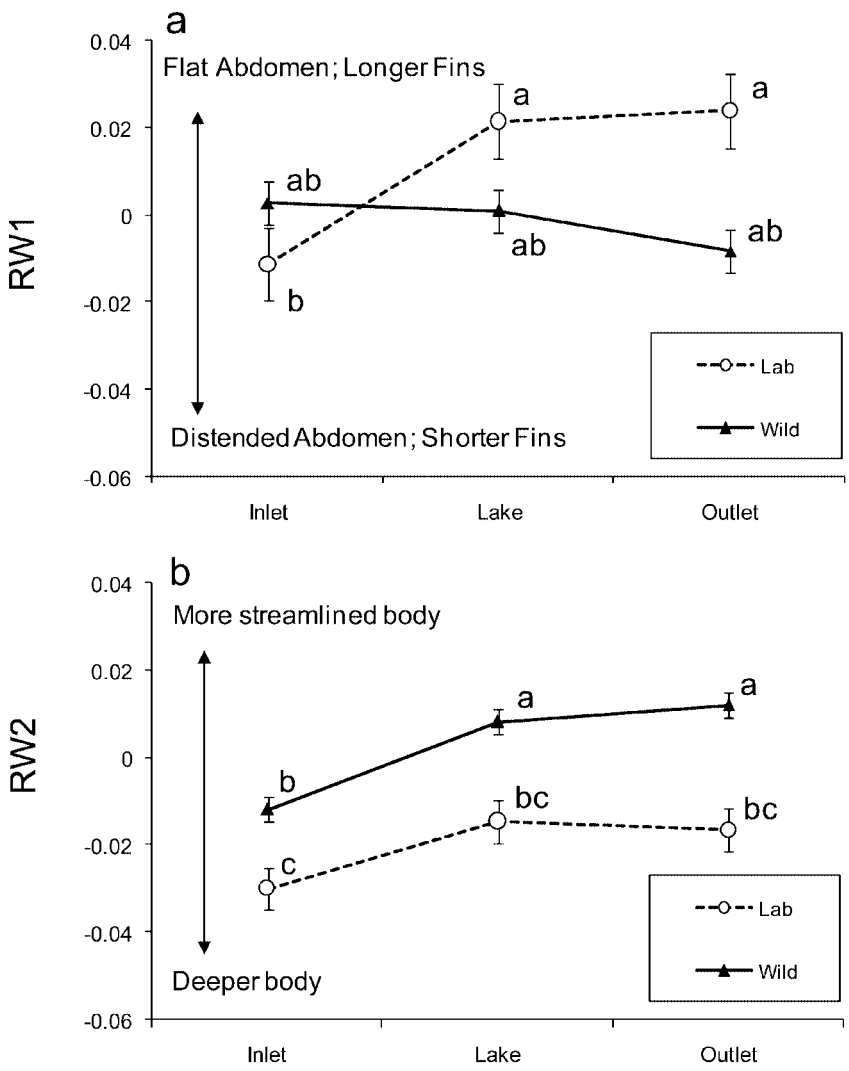

Fig. 4. Variation in body shape among Misty Lake stickleback along RW1 (a) and RW2 (b). Symbols represent RW least-square means (± standard errors) for each combination of ecotype (lake, inlet, outlet) and rearing environment (wild, laboratory). Refer to Fig. 2 for thin plate spline visualizations of variation associated with RW1 and RW2. Superscripts indicate homogeneous subsets based on *post hoc* Tukey tests.

significant (Tukey tests; Fig. 4a), and were not consistent across rearing environments. Another major result was that the body shape of lake and outlet fish was very similar across rearing environments in all analyses (Figs. 3 and 4). Finally, there was no significant sexual dimorphism in body shape (sex: $P > 0.90$ in MANCOVA, $P > 0.80$ in ANCOVA). These results thus confirm that (1) the dramatically deeper body of wild inlet fish relative to wild lake fish has a strong genetic basis, and (2) the *similarity* in body shape between wild lake and wild outlet fish also has a genetic basis.

Table 2. Results of ANCOVA for variation in body shape among Misty Lake stickleback as represented by RW1 and RW2 (see Fig. 2)

Source of variation	d.f.	*F*	*P*	Partial variance explained (%)
RW1				
Rearing environment (RE)	1	4.40	0.038	3.2
Ecotype (E)	2	4.17	0.018	5.9
RE × E	2	9.05	<0.001	11.9
Centroid size (CS)	1	7.28	0.008	5.2
RE × CS	1	18.26	<0.001	12.0
RW2				
Rearing environment (RE)	1	42.87	<0.001	24.2
Ecotype (E)	2	19.72	<0.001	22.7
RE × E	2	1.68	0.19	2.4
Centroid size (CS)	1	7.89	0.006	5.6
RE × CS	1	14.02	<0.001	9.5

Note: For other details and conventions, see the footnote to Table 1. Note that the interaction between rearing environment and ecotype (RE × E) was retained in the model for RW2 even though it was not statistically significant. This was done to allow the calculation of least square means for each of the six rearing environment × ecotype combinations, as illustrated in Fig. 4.

MANCOVA also revealed a substantial environmental component to body shape (Table 1). The most striking pattern here was that laboratory-reared fish had deeper bodies along almost their entire length than did wild-caught fish, a pattern consistent across ecotypes (Fig. 3). ANCOVA of RW2, which captured similar variation, confirmed this result (Fig. 4, Table 2). Again, variation in RW1 was more difficult to interpret, as the overall differences among ecotypes were not statistically significant (Tukey tests; Fig. 4a), and there was no consistent response to the laboratory environment.

In addition to the above main effects of ecotype and rearing environment, MANCOVA revealed a significant interaction between these factors (Table 1). In particular, ANCOVA of RW1 (Fig. 4a) revealed that all ecotypes were similar in the wild but inlet fish had more distended abdomens and shorter fins than lake and outlet fish in the laboratory. In general, however, patterns of body shape variation among ecotypes were consistent across rearing environments, as confirmed by the fact that PCV analysis found no significant differences among phenotypic change vectors in either magnitude or angle (Table 3), and by the lack of any significant interaction between rearing environment and ecotype for RW2 (Table 2, Fig. 4).

Genetic and environmental influences on linear traits

MANCOVA revealed that variation in linear traits among lake and stream stickleback had a significant genetic (ecotype) component (Table 4). Patterns of divergence among ecotypes were similar when visualized along canonical variates from MANCOVA (Fig. 5a) or along principal components from PCVA (Fig. 5b), although trait loadings along CVs and PCs were not identical (Table 6). The most striking pattern that was consistent across analyses was that inlet fish had shorter pelvic and first dorsal spines, and deeper caudal peduncles

Table 3. Results of phenotypic change vector analysis (PCVA) for body shape variation among Misty Lake stickleback

Contrast	Vector difference (magnitude)	*P*	Vector difference (angle)	*P*
Inlet–lake	0.012	0.416	29.3	0.888
Inlet–outlet	0.008	0.451	44.8	0.637
Lake–outlet	0.008	0.384	123.7	0.221

Note: For each pair-wise contrast of ecotypes (e.g. inlet–lake), we tested whether phenotypic divergence was similar in magnitude and angle between the two rearing environments. None of the *P*-values is significant, indicating phenotypic differences among ecotypes are conserved across the rearing environments.

Table 4. Results of MANCOVA for variation in linear trait measurements among Misty Lake stickleback

Source of variation	*F*	d.f.	*P*	Partial variance explained (%)
Rearing environment (RE)	12.81	12, 120	<0.001	56.2
Ecotype (E)	2.72	24, 240	<0.001	21.4
RE × E	3.80	24, 240	<0.001	27.5
Body length (TBL)	62.49	12, 120	<0.001	86.2
RE × TBL	5.21	12, 120	<0.001	18.9

Note: For other details and conventions, see the footnote to Table 1.

Table 5. Results of phenotypic change vector analysis (PCVA) for variation in linear trait measurements among Misty Lake stickleback

Contrast	Vector difference (magnitude)	*P*	Vector difference (angle)	*P*
Inlet–lake	0.048	0.140	16.1	0.724
Inlet–outlet	0.050	0.502	31.2	0.052
Lake–outlet	0.104	**0.016**	96.0	0.055

Note: The single significant *P*-value (**bold**) indicates that the magnitude of morphological difference between lake and outlet fish was clearly different between the rearing environments. Some habitat-related differences in orientation of the vectors connecting outlet and lake/inlet ecotypes are suggested by marginally significant *P*-values.

than did lake fish (Tables 6 and 7, Fig. 5). Some differences were also seen for other traits, but we do not discuss them at length because they were less consistent across analyses, as revealed by very different loadings on the CVs and PCs (Table 6). Another important observation with respect to the linear measurements is that lake and outlet fish differed somewhat in the wild but not at all in the laboratory (Fig. 5). Finally, there was no significant sexual dimorphism in linear traits (sex: $P > 0.40$ in MANCOVA). These results suggest that (1) inlet fish differ genetically from lake and outlet fish in (at least) spine length and

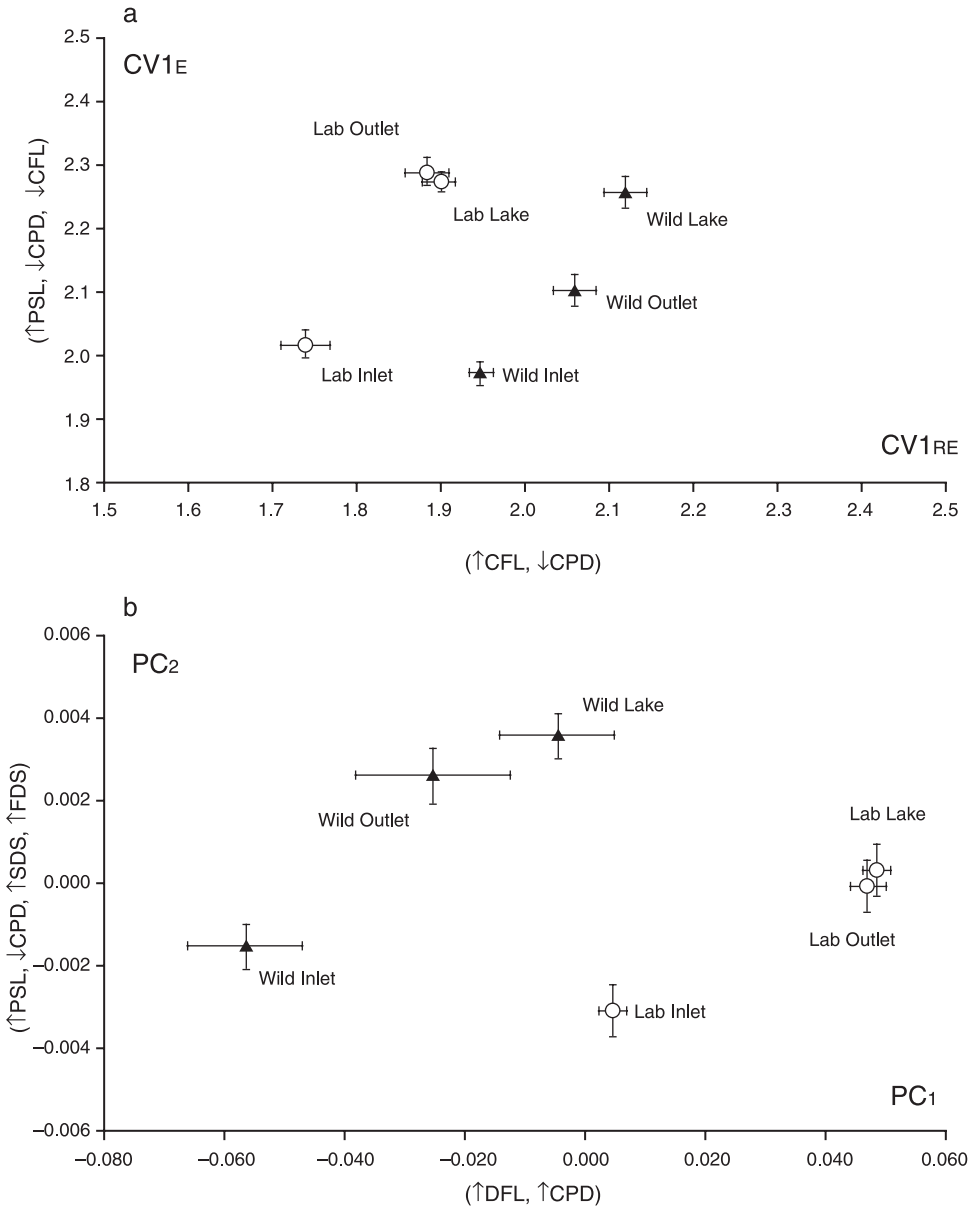

Fig. 5. Variation in linear traits among Misty Lake stickleback. Panel (a) shows results for canonical variates for ecotype ($CV1_E$) and rearing environment ($CV1_{RE}$). Symbols represent mean scores (± standard errors) for each population along each CV. Traits that loaded most heavily onto each CV are indicated (CFL = caudal fin length, CPD = caudal peduncle depth, PSL = pectoral spine length). Note that CV axes are constructed to maximize the phenotypic divergence between groups for a given factor, and do not necessarily represent true phenotypic space. Panel (b) shows results for the first two principal components extracted from LS means, as originally obtained from MANCOVA for each ecotype × rearing environment combination. Individuals were then back-projected on these axes to compute standard errors around the LS means. PC1 explained 92.7% (eigenvalue) of the variation among LS means, while PC2 explained 5.2%. Traits that loaded most heavily onto each PC are indicated (DFL = dorsal fin length, CPD = caudal peduncle depth, PSL = pectoral spine length, SDS = second dorsal spine length, FDS = first dorsal spine length).

Table 6. Loadings of linear trait measurements on canonical variates (CVs from MANCOVA, Fig. 5a) and principal component axes (Fig. 5b) for Misty Lake stickleback

Traits	$CV1_E$	$CV1_{RE}$	PC1	PC2
Jaw length	0.0598	0.651	0.048	−0.005
Eye width	**1.197**	**−1.368**	0.048	−0.007
Pectoral fin width	0.162	−0.321	0.039	−0.002
Pectoral fin length	−0.501	−0.655	**0.066**	−0.011
Pelvic spine length	**1.568**	0.063	0.060	**0.020**
Dorsal fin length	0.308	0.193	**0.086**	−0.013
Anal fin length	1.037	0.559	**0.072**	0.003
Caudal peduncle depth	**−1.593**	**−2.785**	**0.072**	**−0.026**
Caudal peduncle length	0.411	1.004	0.046	0.010
Caudal fin length	**−1.688**	**1.979**	0.035	0.001
First dorsal spine length	0.837	0.142	0.057	**0.020**
Second dorsal spine length	−0.240	**1.055**	0.053	**0.023**

Note: Results are shown for the first canonical axis for ecotype ($CV1_E$) and for rearing environment ($CV1_{RE}$). PCs were generated from principal components analysis performed on the six ecotype LS means obtained from the same MANCOVA. Results are shown for the first two principal components (PC1 and PC2). The four traits that loaded most heavily on each axis are indicated in **bold**.

Table 7. Least-squares means for the linear trait measurements for Misty Lake stickleback

	Laboratory/ inlet	Laboratory/ lake	Laboratory/ outlet	Wild/ inlet	Wild/ lake	Wild/ outlet
Jaw length	3.93	3.69	3.70	4.10	3.97	3.78
Eye width	4.95	4.80	4.96	4.96	4.83	4.71
Pectoral fin width	3.14	3.16	2.97	3.24	3.01	3.32
Pectoral fin length	8.09	7.74	7.73	7.91	7.38	7.99
Pelvic spine length	8.89	9.57	9.64	8.74	9.92	9.62
Dorsal fin length	11.93	11.90	12.12	12.17	11.76	10.93
Anal fin length	9.12	9.80	9.83	9.13	9.45	8.88
Caudal peduncle depth	2.94	2.64	2.64	2.59	2.44	2.46
Caudal peduncle length	9.65	9.28	9.18	9.54	9.76	10.20
Caudal fin length	6.69	6.32	6.32	7.36	6.82	7.07
First dorsal spine	5.66	6.01	5.93	5.86	6.50	6.07
Second dorsal spine	6.01	6.35	6.28	6.15	6.97	6.65

Note: Values shown are least squares means from ANCOVAs with rearing environment, ecotype, and rearing environment × ecotype as main effects, and log-transformed total body length as the covariate. Log-transformed trait values were used in the ANCOVA, so back-transformed means (mm) are shown here. These ANCOVAs were not used for significance testing (see MANCOVA, Table 4); the means shown here are for descriptive purposes only.

caudal peduncle depth, and (2) lake and outlet fish do not differ genetically from each other in linear traits.

MANCOVA revealed that the relative environmental component of variation was stronger for linear measurements (Table 4) than for body shape (Table 1). Consistent across

the analyses, laboratory-reared fish had deeper caudal peduncles than did wild-caught fish (Table 6, Fig. 5). Laboratory-reared fish also showed decreases in caudal fin length and in the length of the first and second dorsal spines (Table 7), although this was not consistent across all analyses. MANCOVA also found a significant interaction between rearing environment and ecotype (Table 4) for linear measurements.

PCVA revealed that this interaction was driven mainly by the lake and outlet populations, which were more different in the wild than in the laboratory (Table 5). Specifically, lake and outlet fish differed in the width of the pectoral fin base, dorsal and anal fin lengths, and caudal peduncle length in the wild, but much less so in the laboratory (Table 7). PCVA also suggested habitat-related changes in the orientation of morphological differences between the outlet and each of the two other ecotypes (the differences in vector angles across rearing environments for outlet–lake and outlet–inlet pairs were marginally significant: $0.05 < P < 0.06$; Table 5).

DISCUSSION

Disentangling the genetic and environmental contributions to phenotypic variation in natural populations is important for understanding the factors that promote or constrain evolutionary diversification (Crispo, 2008). In the Misty Lake system, past inferences about the role of natural selection and gene flow in promoting or constraining adaptive divergence and reproductive isolation have assumed that patterns of phenotypic variation among wild populations have a genetic basis (Lavin and McPhail, 1993; Hendry *et al.*, 2002; Hendry and Taylor, 2004; Moore and Hendry, 2005; Moore *et al.*, 2007). In this study, we tested this assumption by comparing lake, inlet, and outlet stickleback collected from the wild and raised under common conditions in the laboratory. We found that the most striking morphological differences between wild lake and inlet fish were maintained under common-garden rearing, as were the main morphological similarities between wild lake and outlet fish. We also found substantial effects of rearing environment on morphology, but these effects had little influence on the differences between the inlet and lake/outlet ecotypes. We now discuss each of these findings in turn and then consider the implications for gene flow and reproductive isolation in this system.

Genetic and plastic basis of morphological variation

We found striking genetic differences between inlet and lake/outlet stickleback in a number of morphological traits. In the wild, inlet fish had deeper bodies along their entire length and deeper caudal peduncles than did lake and outlet fish. These differences were maintained under common-garden rearing in the laboratory, indicating a genetic basis. Our study thus confirms and extends the results of previous common-garden analyses of body depth in Misty stickleback (Lavin and McPhail, 1993; Hendry *et al.*, 2002), which were based on only a single linear measurement of body depth. Our results further complement recent geometric morphometric studies that have found a genetic basis for body shape in other stickleback populations (Schluter *et al.*, 2004; Albert *et al.*, 2008).

Body shape differences in Misty Lake stickleback likely reflect adaptation to the different foraging environments of lakes versus streams. Stream stickleback feed almost exclusively on benthic macroinvertebrates and forage in structurally complex environments. Lake stickleback, in contrast, often feed on zooplankton in the open water (Berner *et al.*, 2008). The

difference in these foraging modes should favour deep bodies and caudal peduncles in stream stickleback, perhaps to aid burst swimming and manoeuvrability (Walker, 1997), but streamlined (shallow) bodies and narrow caudal peduncles in lake stickleback, perhaps to aid efficiency during sustained swimming (Weihs and Webb, 1983; Webb, 1984; Blake *et al.*, 2005). Paralleling these expectations, empirical studies of swimming performance in both benthic/limnetic and marine/anadromous stickleback pairs show that deeper bodies increase drag, whereas more streamlined bodies enhance endurance during sustained swimming (Taylor and McPhail, 1986; Blake *et al.*, 2005).

We also found strong genetically based differences between inlet and lake/outlet stickleback in traits related to predator defence. Inlet stickleback had shorter dorsal and pelvic spines than did lake and outlet stickleback, and these differences were again maintained in the laboratory. These findings confirm previous work on pelvic spines in the Misty system (Lavin and McPhail, 1993; Hendry *et al.*, 2002), and are consistent with evidence of a genetic basis for spine length in other stickleback populations (Peichel *et al.*, 2001). Pelvic spines are known to protect stickleback against piscivorous predators such as trout, and geographic surveys have found a positive correlation between pelvic spine length and presence of predators (Hagen and Gilbertson, 1972). Our results suggest that lake stickleback are exposed to more predation than are stream stickleback, as is the case for other lake–stream populations in British Columbia (e.g. Moodie, 1972). However, confirmation of this predator-based hypothesis awaits a formal comparison of predation rates in this system.

In the wild, outlet stickleback differed little from lake stickleback in terms of body shape, but were somewhat intermediate between lake and inlet fish for linear measurements. This pattern largely parallels previous findings in the Misty system (Hendry *et al.*, 2002; Moore *et al.*, 2007). We further found that the subtle morphological differences seen in the wild between Misty lake and outlet stickleback largely disappear in a common-garden environment. Phenotypic differences between wild lake and outlet fish may therefore reflect one or more of the following possibilities. First, phenotypic plasticity is a likely explanation, given that many of the traits showed substantial environmental variation (between the wild and the laboratory – see below), and that lake and outlet fish experience very different conditions in the wild (Moore *et al.*, 2007; Berner *et al.*, 2008). Second, selection within a generation can cause morphological differences in the absence of genetic differences, as has been found for some traits in wild outlet stickleback (J.S. Moore and A.P. Hendry, unpublished data). Third, phenotype-specific habitat choice may result in stickleback with more 'stream-like' or more 'lake-like' morphology preferentially occupying the outlet and the lake, respectively. This hypothesis is at least somewhat supported by the fact that lake and stream stickleback show some tendency to avoid the opposite habitat (Hendry *et al.*, 2002; D. Bolnick, unpublished data). Fourth, genotype-by-environment interactions may dictate that any genetic differences seen in the wild are not manifested in the common-garden laboratory environment. Fifth, lake and outlet stickleback may differ genetically in behaviours that result in plastic changes in morphology in the wild. In these last two examples, genetic differences may actually be the cause of the observed morphological differences in the wild – but these are not detectable in a common garden.

Many of the traits we examined were subject to strong plastic effects associated with rearing environment (wild vs. laboratory). Most obviously, laboratory-reared fish had much deeper bodies (along their entire length) than did wild-caught fish, a trend that was consistent across all ecotypes. This plastic response may have arisen from differences in swimming and foraging opportunities between the laboratory and in the wild (e.g. space

constraints in aquaria), but more specific experiments are needed to identify the actual underlying mechanism. We also want to note that since we studied first-generation laboratory-reared fish, we cannot fully exclude the possibility that maternal effects mediated via egg size/quality also contributed to the observed variation. However, such effects are unlikely to have caused the strong patterns seen for adult morphology given that egg sizes do not strongly differ among ecotypes (J.A. Baker *et al.*, unpublished data).

Implications for gene flow and reproductive isolation

Taken together, our results suggest that several traits may show plastic responses to the environment, but that the striking morphological differences between wild Misty lake and inlet stickleback are genetically based. These support the key conclusions of our previous work (Hendry *et al.*, 2002; Moore *et al.*, 2007; Berner *et al.*, 2008; Delcourt *et al.*, in press). First, divergent selection drives adaptive genetic differences between lake and inlet stickleback in the absence of constraining gene flow. Second, high gene flow constrains adaptive genetic divergence between lake and outlet stickleback despite strong divergent selection.

Inferences about how natural selection drives ecologically based reproductive isolation (i.e. how adaptive divergence constrains gene flow) also require an understanding of the genetic and plastic contributions to phenotypic variation (Crispo, 2008). If, for instance, adaptive phenotypic differences have a strong genetic basis, then selection against migrants and hybrids can contribute to reproductive isolation. If, however, phenotypic variation is mostly plastic, reproductive isolation may not evolve (see Introduction). In the present study, we found strong genetically based differences between lake and inlet stickleback in traits important for swimming, foraging, and predator defence. This suggests that migrants and hybrids between these environments/ecotypes would be maladapted – and therefore suffer higher mortality and/or reduced reproductive success. Reduced fitness of migrants and hybrids has been demonstrated for other highly morphologically divergent stickleback populations [benthic/limnetic stickleback (Rundle, 2002)]. In the Misty system, transplant experiments in the wild have provided some evidence for selection against migrants between the lake and the inlet stream (Hendry *et al.*, 2002; K. Räsänen and A.P. Hendry, unpublished data), but further work is needed to clarify the fitness differences between migrants and residents of these two environments. For the lake and outlet populations, we have no concrete evidence of noteworthy phenotypic differences (whether genetic or plastic), and so we conclude that selection against migrants and hybrids would be unlikely. While all studies seem to support the idea that divergent natural selection drives adaptive divergence between lake and stream stickleback, the jury is still out on how this divergence then contributes to reproductive isolation.

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