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Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback

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ABSTRACT

Questions: Does gene flow constrain adaptation in nature? Does spatial variation in selection make it difficult to detect the role of gene flow?

Data description: Variation in the adaptive morphology of threespine stickleback (*Gasterosteus aculeatus*) from multiple sites in each of three environments: Misty Lake, an inlet stream flowing into Misty Lake, and an outlet stream flowing out of Misty Lake. Variation among these same sites in habitat features that influence natural selection.

Search method: (A) Regressions of site means for morphological traits against distance from the lake. (B) Regressions of site means for morphological traits against site means for water flow. (C) Regression of residuals from (B) against distance from the lake.

Conclusion: Gene flow strongly constrains adaptation in the outlet, as evidenced by gradual shifts in morphology from the lake into the outlet, as well as gradual clines along the outlet. Gene flow does not constrain adaptation in the inlet, as evidenced by sharp shifts in morphology from the lake into the inlet, as well as the absence of clines along the inlet. Both selection and gene flow are required to explain adaptive variation within this system.

Keywords: constraints, dispersal, divergent selection, ecological speciation, hybrid zones, migration, parallel evolution.

INTRODUCTION

Natural selection drives the adaptive divergence of populations inhabiting different ecological environments (Endler, 1986; Schluter, 2000). The magnitude of this divergence, however, should be constrained by gene flow between the environments, as shown in numerous theoretical models (e.g. Haldane, 1948; Slatkin, 1973; García-Ramos and Kirkpatrick, 1997; Hendry *et al.*, 2001; Lenormand, 2002) and some empirical studies (e.g. King and Lawson, 1995; Hendry *et al.*, 2002; Saint-Laurent *et al.*, 2003; Hendry and Taylor, 2004; Nosil and Crespi, 2004). Despite this diverse support for each process, the relative importance of natural selection and gene flow to adaptive divergence in nature

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remains controversial (Ehrlich and Raven, 1969; Slatkin, 1987; Blondel *et al.*, 1999; Storfer, 1999; Calsbeek and Smith, 2003). And yet quantifying this role is critical for understanding how biological diversity is generated and maintained, and how it can best be conserved. For example, the translocation of individuals among isolated populations is a common management strategy, but this strategy could reduce the fitness of populations adapted to different environments (Storfer, 1999; Boulding and Hay, 2001; Kawecki and Holt, 2002; Stockwell *et al.*, 2003).

One obstacle to inferring the relative roles of selection and gene flow is that their effects can be difficult to disentangle – that is, variation in adaptive divergence could reflect both processes (Hendry and Taylor, 2004; Garant *et al.*, 2005; Postma and van Noordwijk, 2005). For example, apparent reductions in expected morphological divergence between populations in different environments could reflect stronger gene flow or weaker divergent selection. The analysis of clinal variation provides a potentially powerful tool for disentangling these effects (Haldane, 1948; Slatkin, 1973; Endler, 1977; Lenormand, 2002; Storz, 2002). At its simplest, this approach examines spatial patterns of morphological variation in a species whose distribution crosses a shift in selective environments (e.g. Jain and Bradshaw, 1966; McNeilly, 1968; Endler, 1977; Barton and Hewitt, 1985; Storz, 2002). Gene flow is then inferred to play a constraining role in adaptation when morphology shifts gradually across a sharp transition in the selective environment. We here use clines in morphological traits and habitat features to infer the influence of gene flow and selection on adaptive divergence between threespine stickleback (*Gasterosteus aculeatus* L.) populations in lakes and streams.

Lake and stream stickleback

Divergent population pairs of threespine stickleback have been described for a number of watersheds in the northern hemisphere (McPhail, 1994; McKinnon and Rundle, 2002). Many of these pairs evolved independently since the last glaciation (approximately 10,000 years ago), and now show substantial adaptive divergence and reproductive isolation (Schluter and McPhail, 1992; Lavin and McPhail, 1993; Thompson *et al.*, 1997; Taylor and McPhail, 1999; McKinnon and Rundle, 2002). Mainly for these reasons, the threespine stickleback has become a popular species for studying adaptation and speciation in nature (Schluter, 2000; McKinnon and Rundle, 2002). Our work focuses on parapatric lake–stream population pairs, which are found in a number of different watersheds (Moodie, 1972a,b; Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Hendry *et al.*, 2002; Hendry and Taylor, 2004).

Lake and stream stickleback differ in a suite of morphological traits believed to be adaptive for their respective environments. Most obviously, stream fish have fewer gill rakers and deeper bodies than lake fish, differences that have an additive genetic basis (Moodie, 1972a,b; Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Hendry *et al.*, 2002). The difference in gill rakers is probably adaptive because more numerous gill rakers are better suited for feeding on zooplankton, which predominate in lakes, whereas fewer gill rakers are better suited for feeding on benthic macro-invertebrates, which predominate in streams (see references in Hendry *et al.*, 2002). The difference in body depth is probably adaptive because streamlined (shallow) bodies are better suited for sustained swimming, which likely predominates in lakes, whereas robust (deep) bodies are better suited for burst swimming and precise manoeuvring, which likely predominate in streams (see references in Hendry *et al.*, 2002). Stream and lake stickleback can also differ in several defensive armour traits, such as pelvic spines and lateral plates (Moodie, 1972a,b; Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Hendry *et al.*, 2002), although the direction of divergence between lake and stream populations for these traits is not consistent across systems (Hendry

and Taylor, 2004). We here focus on morphological variation within the Misty Lake system, an archetypal lake–stream pair (Lavin and McPhail, 1993; Thompson *et al.*, 1997; Hendry *et al.*, 2002).

The Misty Lake system

Our study was conducted in Misty Lake and its inlet and outlet streams (Fig. 1). Lake and inlet stickleback in this system differ morphologically, with outlet fish being intermediate (Fig. 2) (Lavin and McPhail, 1993; Hendry *et al.*, 2002). Hendry *et al.* (2002) advanced the hypothesis that the intermediacy of outlet fish was the result of high levels of gene flow from the lake. Supporting this hypothesis, gene flow as estimated by both microsatellites and mtDNA was considerably greater between the lake and the outlet than between the lake and the inlet. And yet an alternative explanation remained viable: selection for a 'stream-like' form may be weaker in the outlet than in the inlet. If so, the morphological intermediacy of outlet fish could be the result of weaker selection, which might then allow higher gene flow from the lake because less ecologically dependent reproductive isolation would be expected to evolve (Schluter, 2000; Hendry, 2004).

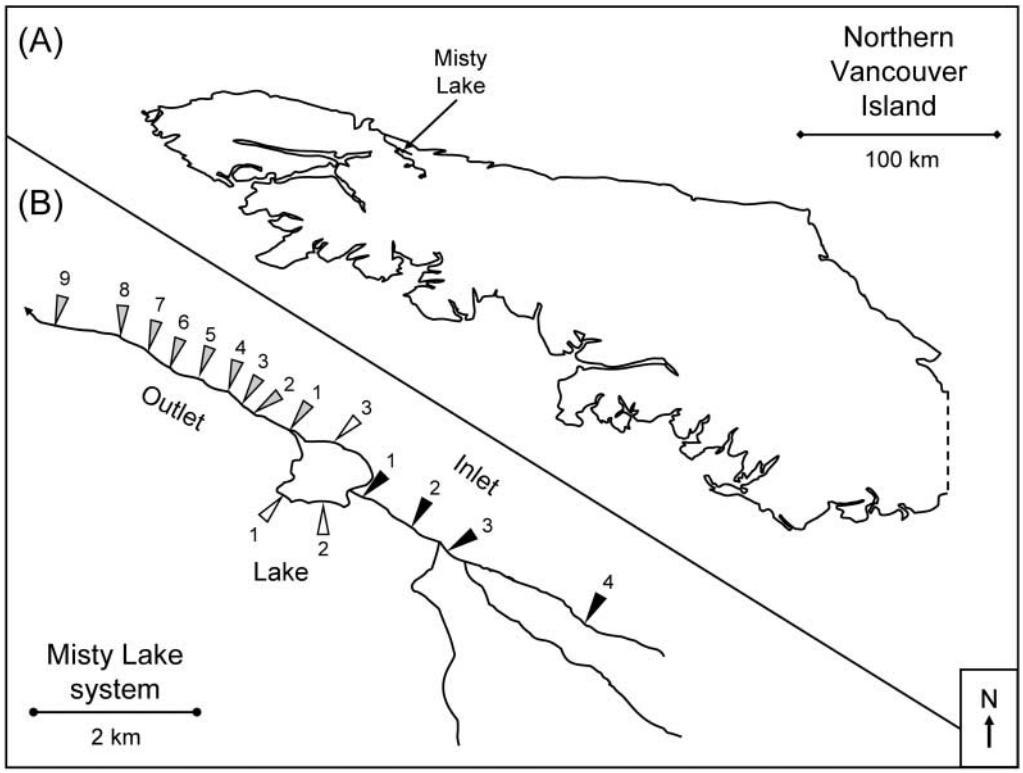

Fig. 1. (A) Location of the Misty Lake system on northern Vancouver Island, British Columbia, Canada, with all other drainages omitted. (B) Locations of sampling sites within the Misty Lake system.

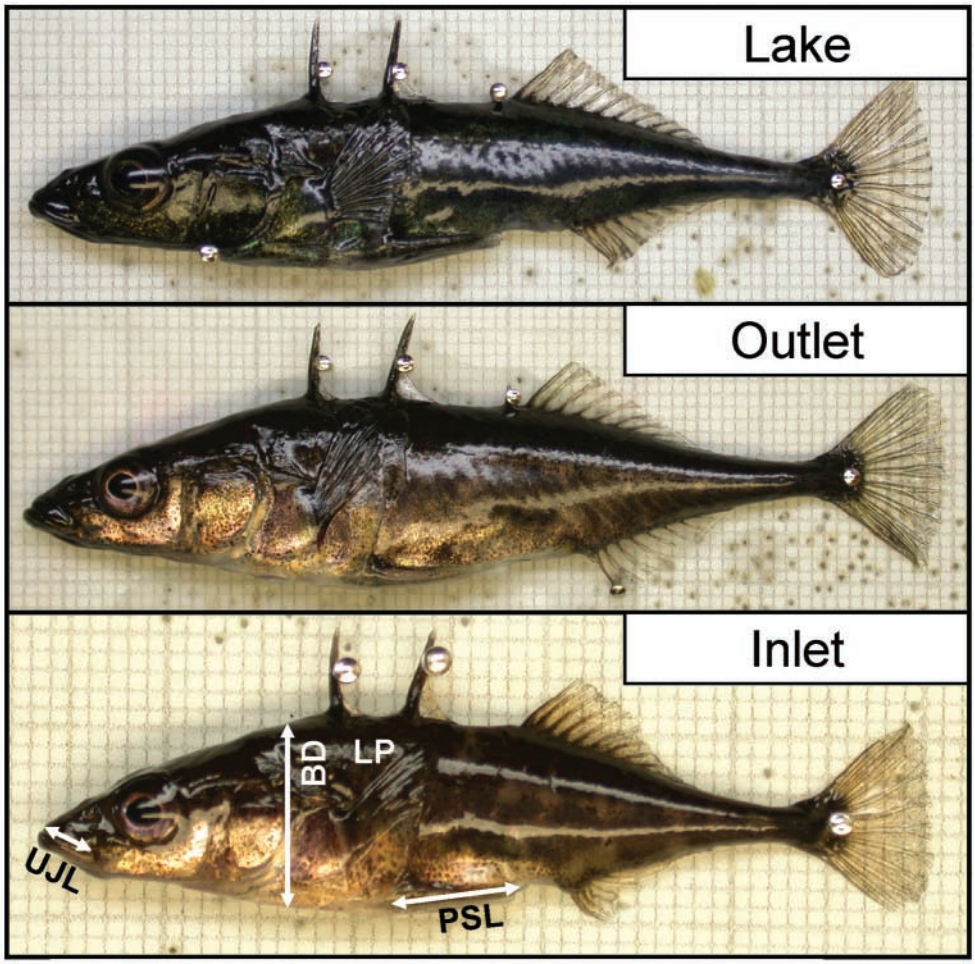

Fig. 2. Representative lake (from Lake site 2), inlet (from Inlet site 2) and outlet (from Outlet site 4) stickleback from the Misty Lake system. The fish are scaled to a common body length so as to better illustrate the shape differences (the small squares in each image are 1 mm^2). Superimposed on the inlet fish are the locations of the lateral plates (LP), the upper jaw length measurement (UJL), the body depth measurement (BD) and the pelvic spine length measurement (PSL). The other traits cannot be illustrated in the lateral plane.

Our goal in the present study was to better delineate the roles of gene flow and selection in shaping morphological variation within the Misty Lake system. Specifically, we examined how morphological traits and habitat features in streams covary with each other and with distance from the lake. In an earlier study adopting the clinal approach for stickleback, Bell and Richkind (1981) were able to infer that gene flow between predation regimes constrains divergence in armour traits. Our study extends this basic approach by examining two different stream environments, an inlet and an outlet. Comparing clines between these environments is informative because physical dispersal by lake stickleback, which are much

more abundant that stream stickleback, should be considerably higher into the outlet than into the inlet (Hendry *et al.*, 2002; Hendry and Taylor, 2004). As a further extension, we measured ecologically relevant habitat features at the different stream sites, which allowed us to account for some of the potential variation in selection.

The overall goal of our research on lake/stream stickleback is to elucidate interactions between selection, gene flow, adaptation and reproductive isolation. The present study advances this effort by demonstrating that variation in morphological divergence is clearly influenced by both gene flow and selection.

MATERIALS AND METHODS

Study sites and habitat features

Misty Lake (50°36′32″N, 127°15′46″W) is a small (surface area = 35.6 ha) and shallow (mean depth = 1.7 m; maximum depth = 6.1 m) lake located in the Keogh River system on northern Vancouver Island, British Columbia (Fig. 1). We studied three sites in the lake, four sites in the inlet and nine sites in the outlet (Fig. 1; Table 1). Only four sites were sampled in the inlet and three sites in the lake because spatial variation in morphology is low within these two environments (Hendry *et al.*, 2002; present study). All sample sites were more than 10 km from the ocean and no anadromous stickleback were present.

Habitat features were measured at all stream sites, except that time constraints precluded doing so for site 4 in the inlet. All data were collected between 23 May and 4 June 2004, a period during which water levels remained relatively stable because snow melt had ceased and rain was minimal. At each site, we established 11 transects evenly spaced every 5–10 m along the stream. Spacing was constant at each site but varied among sites depending on the area from which stickleback were collected. At the centre of each transect, we measured the wetted width of the channel (m). At each of three equidistant points across each transect, we measured water depth (cm) and water flow ($m \cdot s^{-1}$) (Swoffer model 2100 flow meter, Seattle, WA; impeller positioned 60% of the distance from the substrate to the surface). These habitat measurements parallel those in Hendry and Taylor (2004).

Morphology

We used unbaited minnow traps to collect stickleback in July 2003 and May–June 2004. When less than 30 stickleback were collected at a site, all were retained for analysis. When more than 30 were collected, a subset of 30 were haphazardly selected and retained for analysis. Fish were killed with an overdose of tricaine methanesulphonate (MS-222) and preserved in 95% ethanol. Ethanol was chosen as the preservative to facilitate genetic analysis, as well as comparison with our previous work (Hendry *et al.*, 2002; Hendry and Taylor, 2004). A minimum of one month after the collections, morphological measurements were made by a single person (J.-S. Moore), who was blind to the origin of the fish. Measured traits were those examined in our previous work (Hendry *et al.*, 2002; Hendry and Taylor, 2004): body length (tip of upper jaw to end of hypural plate), body depth (anterior end of dorsal plate immediately in front of first dorsal spine to bottom of pelvic girdle, perpendicular to the lateral line), pelvic spine length (insertion to tip of left spine), upper jaw length (tip of jaw to end of maxilla), number of gill rakers (left side of first gill arch), number of lateral plates (on the left side) and pelvic girdle width (at its widest) (Fig. 2).

Stickleback body size is influenced by growth conditions (i.e. phenotypic plasticity), and so we do not attempt to interpret clinal variation in body length. We also statistically removed (Reist, 1986) the effects of body size from traits with which it is correlated: body depth, upper jaw length, pelvic spine length and pelvic girdle width (see also Hendry *et al.*, 2002; Hendry and Taylor, 2004). We first $\log_{10}$-transformed all measurements. We then used analysis of covariance (ANCOVA) to test for heterogeneity of slopes among sites – that is, the site-by-length interaction term in ANCOVA. Slopes were homogeneous for all traits (all $P > 0.078$) except for pelvic spine length ($P = 0.016$). The heterogeneity for spine length was the result of only a single site (outlet 6), and so we assumed the typical case was the lack of an interaction. We next removed the interaction term from the analyses of covariance to allow the calculation of common slope coefficients (b) for each trait: body depth (1.075), upper jaw length (1.291), pelvic spine length (0.634) and pelvic girdle width (1.986). Allometric standardization of each trait to the mean body size of all collected fish (51.7 mm) was then performed as $M_{std} = M_o(51.7/L_o)^b$, where M is trait size, L is body length, b is the ANCOVA slope with the interaction term removed, and the subscripts 'std' and 'o' refer to standardized and observed measurements (neither $\log_{10}$ transformed).

We also calculated a composite multivariate index of morphological variation based on eigenvalues from discriminant functions (e.g. Lu and Bernatchez, 1999; Hendry *et al.*, 2002; Hendry and Taylor, 2004; Nosil and Crespi, 2004). This index was based on shared variation in gill raker number, standardized body depth and standardized pelvic spine length. The other traits were excluded because (1) their genetic basis and functional interpretation is less clear, and (2) they do not necessarily differ between archetypal lake–stream pairs (Moodie, 1972a,b; Reimchen *et al.*, 1985; Lavin and McPhail, 1993; Hendry *et al.*, 2002; Hendry and Taylor, 2004). We also sexed all of the fish by dissection and examination of the gonads. Mature males have small and dark testes, whereas mature females have larger and unpigmented ovaries. Individuals for which sex could not be unambiguously determined were excluded from the analyses (except where noted), a conservative approach that reduced samples sizes below the 30 originally measured for each site.

Statistical analysis and interpretation

Variation in each morphological trait (size-standardized as necessary) and the first discriminant function was examined using analysis of variance (ANOVA) and linear regression. First, we used sites with collections in both years (lake site 1, inlet site 3, and outlet sites 4 and 1) to test for inter-annual variation. We employed a three-way ANOVA where the factors were site (fixed), sex (fixed) and year (random). The main effect of year was non-significant for all traits (all $P > 0.462$), and interactions between sex and year were non-significant for all traits (all $P > 0.1$) except body length ($P = 0.032$). Owing to the lack of inter-annual variation at these four sites, we pooled samples from different years and ignored year effects in all subsequent analyses. We nevertheless present year-specific values in the tables and figures so that the reader can verify the consistency between years. Second, we tested for sexual dimorphism with a two-way ANOVA including all sites (site and sex were fixed effects). Sex had significant effects on some of the traits: body length ($P < 0.001$), body depth ($P < 0.001$), upper jaw length ($P < 0.001$) and pelvic girdle width ($P = 0.002$). Sex generally did not interact significantly with site ($P > 0.12$), except for upper jaw length ($P = 0.001$) and pelvic girdle width ($P = 0.044$). Because sex had a significant effect on

morphology, all subsequent analyses considered the sexes separately. Third, we tested for differences between sample sites with one-way analyses of variance for each trait in each sex. Fourth, we examined clines in trait values by regressing site-means for each trait against distance from the lake.

Habitat features might differ between the inlet and outlet, and might vary with distance from the lake. If so, they might influence selection on morphology. We tested for spatial variation in habitat features with one-way analyses of variance followed by Tukey tests. We tested for effects of habitat on morphology by regressing mean trait values against mean values for habitat. To reduce the possibility of spurious correlations, we focused on only two traits (standardized body depth and gill raker number) and only one habitat feature (water flow). The two traits were chosen because they have an additive genetic basis (Hendry *et al.*, 2002), differ consistently in direction between lakes and streams (Hendry and Taylor, 2004), and should be under selection related to water flow. Water flow should influence selection on gill raker number because this trait influences foraging opportunities on benthic versus limnetic prey, with the relative abundance of the latter decreasing in flowing water (see references in Hendry *et al.*, 2002). Water flow should influence selection on body depth because this trait influences aspects of swimming performance (Walker, 1997; and see references in Hendry *et al.*, 2002). To account for these possible effects, we calculated residuals from regressions of site means for standardized body depth and gill raker number on site means for water flow. This regression included all stream sites, both outlet and inlet. We then regressed these residuals against distance from the lake separately for the inlet and outlet sites.

If gene flow constrains adaptive divergence, we would expect these analyses to yield two spatial patterns. First, mean trait values in streams should become increasingly divergent from lake values with increasing distance from the lake. Second, residuals from regressions of mean trait values on mean water flow should decrease with increasing distance from the lake. This last prediction arises because the effect of gene flow on constraining adaptation to water flow should decrease with increasing distance from the lake.

RESULTS

Morphology

All of the morphological traits (four of them size-standardized as above) varied among sites within each sex (all $P < 0.008$). In general, lake and inlet fish were at the extremes and outlet fish were intermediate but more similar to lake fish (Table 1). Several of the traits showed significant trends in the outlet with increasing distance from the lake: (1) pelvic spine length decreased for females ($P = 0.002$) but not for males ($P = 0.621$), (2) gill raker number decreased for females ($P = 0.052$) but not for males ($P = 0.469$), and (3) upper jaw length decreased for females ($P = 0.005$) but not for males ($P = 0.357$). None of the other traits showed statistically significant trends with distance from the lake: body depth (females: $P = 0.146$; males: $P = 0.738$), lateral plate number (females: $P = 0.093$; males: $P = 0.561$), and pelvic girdle width (females: $P = 0.146$; males: $P = 0.480$). In all cases, however, the qualitative trend was for outlet fish near the lake to closely resemble lake fish but for outlet fish farther from the lake to increasingly deviate from the lake phenotype in the direction of the inlet phenotype. If the above analyses were performed when the sexes were pooled (including fish that could not be sexed), the same qualitative trends were present for all traits, although statistical significance again varied: body depth ($P = 0.226$), gill raker

number ($P = 0.465$), pelvic spine length ($P = 0.010$), lateral plate number ($P = 0.093$), upper jaw length ($P = 0.103$) and pelvic girdle width ($P = 0.252$).

For the multivariate index of morphology with effects of body size removed, the first canonical function explained 94.7% of the variation for females and 92.8% for males, whereas the second explained only 3.7% for females and 4.3% for males. We therefore report results for the first function only, which had standardized loadings for females of body depth = −0.684, gill raker number = 0.526 and pelvic spine length = 0.422; and for males of body depth = −0.693, gill raker number = 0.564 and pelvic spine length = 0.459. Clinal variation in the multivariate index generally mirrored that for the three traits on which it was based. Group centroids were generally similar for lake fish and outlet fish near the lake, strikingly different for inlet fish, and intermediate for outlet fish far from the lake, although this last group was still more similar to lake fish than to inlet fish (Table 1; Fig. 3). Group centroids were significantly correlated with distance from the lake for females ($P = 0.003$) but not for males ($P = 0.516$). If these analyses were performed when both sexes were pooled, the trend was similar and significant ($P = 0.027$).

Habitat and its effects on morphology

All habitat features varied among sites (all $P < 0.001$; Table 2), and some clinal variation was evident in the outlet, with sites closer to the lake being more lake-like (i.e. deeper and slower flow). However, the habitat clines were abrupt, with most change occurring between outlet site 3 and outlet site 6, whereas the morphological clines were typically gradual across the entire range of outlet sites. As expected, mean water flow was correlated, at least marginally, with mean body depth for females ($P = 0.067$) and males ($P = 0.052$), and perhaps even with mean gill raker number for females ($P = 0.081$) and males ($P = 0.224$). In the outlet, residuals from these regressions converged on zero with increasing distance from the lake for both body depth (females: $P = 0.034$; males: $P = 0.005$) and gill raker number (females: $P = 0.019$; males: $P = 0.01$). This is the pattern expected if sites near the lake are less able to achieve the optimum for these traits with respect to water flow. Residuals in the inlet did not show any significant trends with distance from the lake (Fig. 4): body depth (females: $P = 0.790$; males: $P = 0.995$) and gill raker number ($r^2 = 0.284$, $P = 0.664$). These results suggest that gene flow constrains adaptation in the outlet, particularly near the lake, but not in the inlet (although the number of sites was also fewer in the inlet than in the outlet).

DISCUSSION

Few studies have considered the joint effects of selection and gene flow on adaptation. On the one hand, most studies of adaptive divergence have investigated the role of selection, but did not consider gene flow. On the other hand, most studies of gene flow have not considered its effects on adaptive divergence. The relatively few studies that have attempted this latter task typically have found that gene flow can indeed have substantial effects on adaptation (e.g. King and Lawson, 1995; Hendry *et al.*, 2002; Calsbeek and Smith, 2003; Saint-Laurent *et al.*, 2003; Nosil and Crespi, 2004). Even these latter studies, however, did not also consider variation in selection. This lack of integration is unfortunate because adaptation likely depends in complex ways on the interaction between selection and gene flow (Hendry and Taylor, 2004; Garant *et al.*, 2005; Postma and van Noordwijk, 2005). The present study allowed some insight into this interaction.

Table 1. Average morphological measurements for stickleback from different sites in the Misty Lake system

Site	Year	Distance from the lake (m)	*n*	Body length	Body depth	Pelvic spine length	Upper jaw length	Girdle width	Number of gill rakers	Number of lateral plates	Multivariate morph. index
Outlet 9	2004	2330	5/6	71.2/64.5	10.7/10.6	8.3/8.9	3.1/3.9	3.0/2.7	18.4/20.2	6.6/6.3	−0.714/1.062
Outlet 8	2004	1780	13/8	63.8/59.9	10.3/10.5	8.9/9.2	3.3/3.9	2.9/2.8	19.2/18.8	6.2/5.9	0.771/0.781
Outlet 7	2003	1560	9/13	66.9/60.5	10.4/11.1	9.2/9.0	3.3/3.7	2.8/2.9	18.8/19.2	6.2/6.6	0.792/−0.313
Outlet 6	2003	1220	8/15	66.5/62.7	10.4/10.7	8.8/9.1	3.6/3.9	3.0/2.7	18.3/19.1	6.8/6.9	0.205/0.521
Outlet 5	2003	985	7/21	63.7/59.3	10.6/10.9	9.1/9.3	3.5/3.8	2.9/2.7	19.0/19.2	6.1/6.5	0.410/0.306
Outlet 4	2003	845	17/10	60.0/54.2	10.7/10.7	9.1/9.4	3.5/3.9	3.0/2.8	18.4/19.2	6.0/6.5	−0.010/0.740
Outlet 4	2004	845	8/14	61.9/53.4	10.4/10.8	9.3/8.9	3.3/3.8	2.9/2.8	18.8/18.7	6.3/6.3	1.008/−0.028
Outlet 3	2003	530	11/14	54.2/51.9	10.4/10.6	9.5/9.2	3.6/4.4	2.9/2.8	19.3/19.3	6.3/5.9	1.350/0.797
Outlet 2	2003	300	10/9	58.1/56.2	10.4/10.8	9.2/8.8	3.6/4.0	2.8/3.1	19.1/18.7	6.2/6.0	0.907/−0.256
Outlet 1	2003	0	9/6	57.3/55.3	10.2/10.2	9.4/9.1	3.5/4.0	2.8/3.0	19.8/20.0	6.1/6.2	1.867/1.672
Outlet 1	2004	0	7/11	64.3/56.2	10.2/10.3	9.8/9.4	3.5/3.8	2.9/2.5	20.1/19.4	5.9/6.4	2.486/1.334
Lake 1	2003	—	7/20	59.1/58.0	10.2/10.4	9.3/9.4	3.5/4.0	2.9/2.8	19.7/19.6	6.4/6.3	1.665/1.334
Lake 1	2004	—	3/19	67.4/56.6	10.6/10.6	9.8/9.3	3.5/4.1	3.0/2.7	20.0/19.7	6.3/6.4	1.543/1.164
Lake 2	2003	—	8/20	56.0/55.5	10.2/10.4	9.5/9.6	3.4/4.0	2.6/2.7	19.1/19.0	6.1/6.3	1.738/1.292
Lake 3	2004	—	16/6	61.6/60.2	10.1/10.1	9.4/9.4	3.4/3.8	2.7/2.6	19.9/20.2	6.1/5.7	2.083/2.160
Inlet 1	2003	60	9/12	55.7/50.7	11.3/11.4	8.2/8.0	3.5/4.4	3.5/3.4	17.4/17.2	7.0/6.8	−2.321/−2.537
Inlet 2	2003	460	8/10	55.6/51.2	11.4/11.8	8.5/8.5	3.4/4.5	3.7/3.7	17.1/16.7	6.9/6.5	−2.571/−2.939
Inlet 3	2003	960	8/4	49.1/49.8	11.4/11.6	8.3/8.0	4.0/4.5	3.7/3.4	17.3/17.5	6.4/6.8	−2.623/−2.624
Inlet 3	2004	960	13/10	50.4/47.4	11.1/11.7	8.2/8.1	3.4/4.3	3.9/3.6	16.7/17.8	6.5/6.5	−2.358/−2.515
Inlet 4	2004	2600	13/12	51.2/49.3	11.8/12.1	8.2/7.7	3.7/4.4	4.0/3.7	15.8/15.7	6.7/6.8	−4.136/−4.512

Note: Means for females and males are presented separately (i.e. female/male). Body depth, pelvic spine length, upper jaw length and pelvic girdle width were allometrically standardized to a common body length of 51.7 mm. The multivariate morphological index represents group centroids for the first discriminant function based on standardized body depth, standardized pelvic spine length and the number of gill rakers.

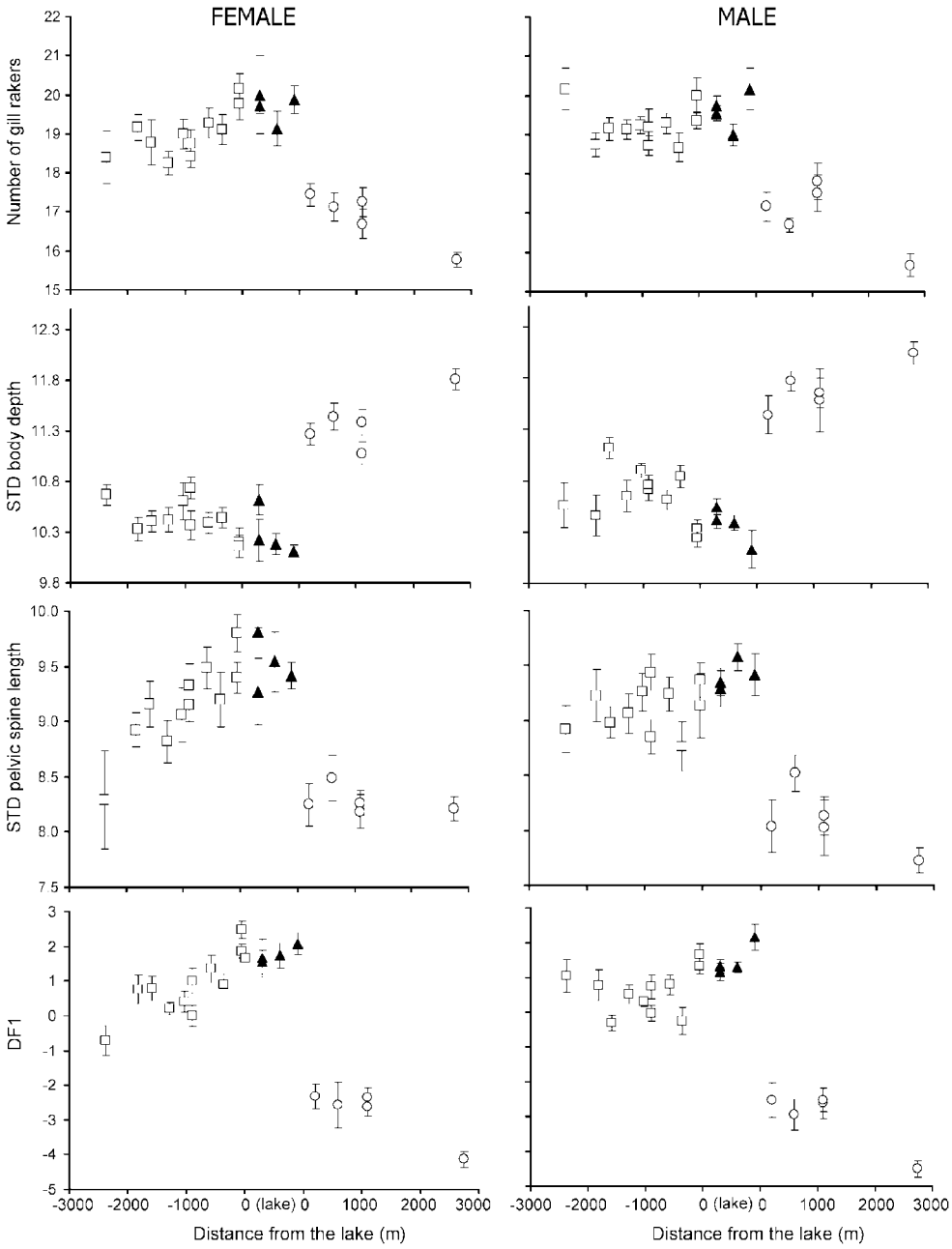
FEMALE
MALE
Number of gill rakers
STD body depth
STD pelvic spine length
DF1
Distance from the lake (m)
Distance from the lake (m)
0 (lake) 0
0 (lake) 0

Table 2. Average values for habitat features at each stream site in the Misty Lake system

Site	Water flow ($m \cdot s^{-1}$)	Water depth (cm)	Stream width (m)
Outlet 9	0.38^{c}	20.2^{a}	$6.5^{a,b}$
Outlet 8	0.17^{b}	22.7^{a}	$6.8^{a,b}$
Outlet 7	0.01^{a}	$34.7^{a,b}$	$9.6^{b,c}$
Outlet 6	0.17^{b}	18.8^{a}	4.3^{a}
Outlet 5	0.00^{a}	$46.8^{a,b,c}$	5.0^{a}
Outlet 4	0.00^{a}	71.0^{c}	$6.1^{a,b}$
Outlet 3	0.00^{a}	$122.0^{d,e}$	16.6^{d}
Outlet 2	0.00^{a}	$139.4^{d,e}$	$9.8^{b,c}$
Outlet 1	0.00^{a}	149.7^{e}	$12.9^{c,d}$
Inlet 1	$0.08^{a,b}$	$54.1^{b,c}$	$6.4^{a,b}$
Inlet 2	0.01^{a}	$30.5^{a,b}$	$6.3^{a,b}$
Inlet 3	0.00^{a}	119.3^{d}	$6.3^{a,b}$

Note: Homogenous subsets based on Tukey tests are indicated with letter superscripts.

Within the Misty Lake system, our results suggest that gene flow does not influence adaptation in the inlet but has a strong influence in the outlet, particularly near the lake. Gene flow has little influence in the inlet because the morphological transition from the lake into the inlet is very dramatic and abrupt (Fig. 3) and because morphological clines appear absent within the inlet (although the farthest site from the lake was more divergent than the others). Thus, morphological divergence between lake and inlet fish probably closely matches the strength of divergent selection caused by ecological differences between the two environments. This interpretation is consistent with previous evidence (Hendry *et al.*, 2002) that (1) gene flow is very low between the lake and the inlet (based on mtDNA and microsatellites), (2) lake and inlet fish appear locally adapted (based on transplant experiments), and (3) lake fish do not move upstream when placed in the inlet (based on mark–recapture).

In contrast, gene flow has a strong influence in the outlet. First, the morphological transition from the lake into the outlet is very gradual, with outlet fish near the lake closely resembling lake fish. Second, outlet stickleback are characterized by a gradual increase in morphological divergence from lake fish with increasing distance from the lake (Fig. 3). These patterns are exactly as expected when gene flow is high across a shift in selection

Fig. 3. Variation in morphology for female (left-hand panels) and male (right-hand panels) threespine stickleback in the Misty Lake system. Shown are means for a given site (bars represent standard errors), with two points at a given site indicating samples from multiple years. The black triangles represent lake samples, the white circles represent inlet samples, and the white squares represent outlet samples. The x axis shows geographical distance from the lake for each sampling location, with negative values used for the outlet and positive values used for the inlet. The y axis scales are identical for females and males for a given trait. 'STD' refers to allometrically standardized traits and 'DF1' refers to group centroids for the first discriminant function, which was based on gill rakers, standardized body depth and standardized pelvic spine length.

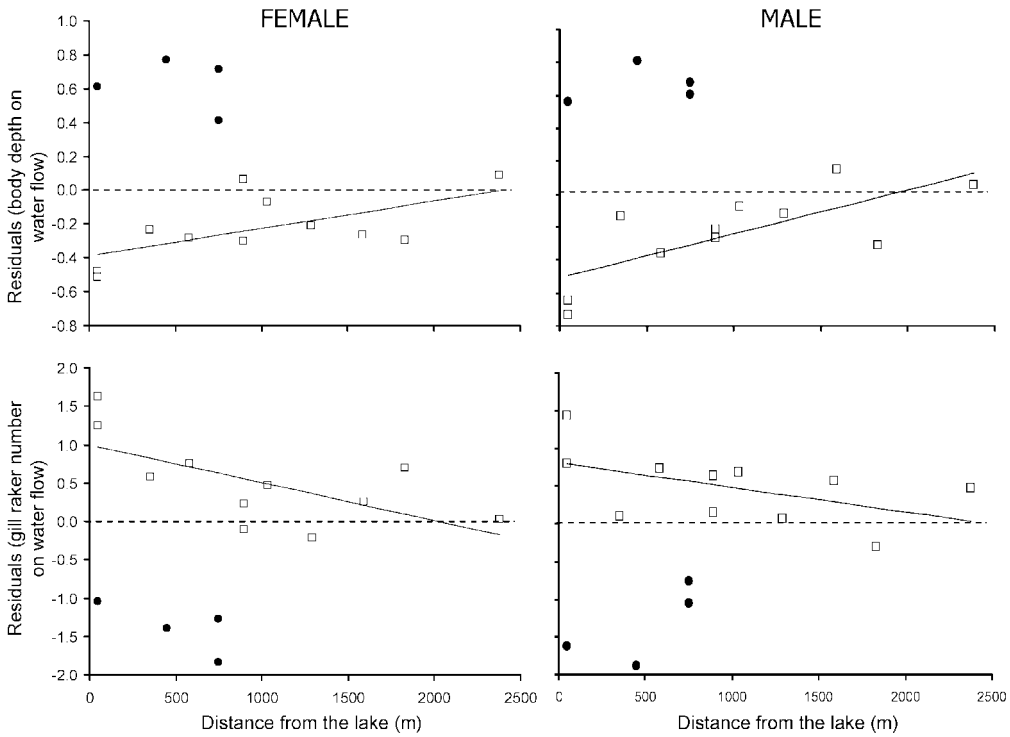

Fig. 4. Among-site variation in standardized body depth and the number of gill rakers for female (left-hand panels) and male (right-hand panels) threespine stickleback, after controlling for variation in water flow. Symbols (black circles for inlet, white squares for outlet) are residuals from regressions of site means for each trait on site means for water flow. The x axis show geographical distance from the lake. The y axis scale is identical for females and males for a given trait. Trend lines show ordinary least-squares relationships in the outlet.

(e.g. Endler, 1977). Although morphological clines in the outlet were in the expected direction for all traits (greater divergence with increasing distance from the lake), not all of the clines were significant. This is to be expected, however, because some of the traits likely experience weaker divergent selection, and because statistical power was limited by the moderate number of sampling sites.

A possible alternative to the constraining role of gene flow in the outlet is a gradual cline in selection. This alternative is worth considering because outlet sites closest to the lake might be most similar to the lake (e.g. deeper water, more zooplankton). Indeed, we found that the two sites closest to the lake were the most lake-like, as characterized by considerably deeper and slower water than the other sites (Table 2). This pattern may play some role in the gradual morphological cline nearest the lake. However, the habitat then shifts dramatically after the third outlet site (Table 2), whereas the morphological cline remains gradual (Fig. 3). Furthermore, when we take habitat differences into account by calculating residuals from regressions of morphology on water flow, deviations from expected stream-like morphology decrease with increasing distance from the lake. We

conclude that gene flow constrains adaptation in the outlet, a result consistent with previous genetic evidence that gene flow is high between the lake and the outlet (Hendry *et al.*, 2002; Hendry and Taylor, 2004).

But is the morphological intermediacy of outlet fish entirely the result of gene flow, or is variation in selection also important – that is, is the selective environment in the outlet intermediate between the lake and inlet? Here, the most interesting result is that outlet stickleback are still very different from inlet stickleback even at our most distant sampling site, 2.3 km from the lake. We can see two possible explanations for this result. One is that high gene flow may occur even over very large distances in the outlet, thus constraining adaptation even far from the lake. This interpretation is supported by several lines of evidence. First, the downstream flow of water might disperse stickleback over long distances, particularly during floods. Hagen (1967) found that almost half of the stream stickleback released at a specific site moved at least 100 m from that site within 30 days, with no striking upstream or downstream bias. Lake stickleback, which would contribute the most to maladaptive gene flow, probably move considerably farther, as many were found to move downstream when placed into the Misty inlet (Hendry *et al.*, 2002). Second, gene flow as measured by microsatellites is high between the lake and outlet site 4 (Hendry *et al.*, 2002), which is already 0.8 km from the lake. Third, stickleback are quite rare in outlet sites 8 and 9, as well as farther downstream (unpublished data), suggesting that gene flow from the lake will not be counterbalanced by large outlet populations farther from the lake (*sensu* Kawecki and Holt, 2002).

An alternative explanation for morphological intermediacy of outlet fish far from the lake is that divergent selection from the lake is weaker in the outlet than in the inlet. If so, outlet fish far from the lake may be well adapted even though they appear less 'stream-like' in their morphology than do inlet fish. Arguing against this possibility, the outlet sites farthest from the lake (sites 8 and 9) are even shallower and faster than the inlet sites (Table 2), and have relatively few stickleback (unpublished data). These results suggest that outlet fish may not be fully adapted even far from the lake, at least to the extent that water depth and flow reflect selection for stream-type morphology. Of course, it remains possible that we did not measure the important selective factors, such as food types, food availability or predation. Future work should quantify these factors and also estimate gene flow among sites in the inlet and outlet. At present, we conclude that the morphological intermediacy of outlet fish is determined jointly by divergent selection, which favours increasingly stream-like forms, and gene flow, which prevents convergence of outlet fish on the extreme form seen in the inlet.

An interesting additional pattern was that morphological clines in the outlet were steeper and more consistent for females than for males (Fig. 3). Theory would suggest that steeper clines might result from lower dispersal or stronger divergent selection (e.g. Endler, 1977). Although sex-biased dispersal is widespread in nature (Greenwood, 1980; Dobson, 1982), it has not been studied in threespine stickleback. One factor that might act against male-biased dispersal is that males construct nests and then defend them, whereas females wander more widely during breeding. It remains possible, however, that males disperse more as juveniles or while searching for nest sites. With respect to sex-biased selection, Reimchen and Nosil (2004) have shown that male and female stickleback in lakes experience different patterns of selection. Whether or not a similar effect occurs in streams is unknown. Further work will be required to determine the reason for differences in clinal variation between males and females.

Implications

Adaptive divergence between populations is best explained through an understanding of both selection and gene flow. The importance of selection is widely accepted (Schluter, 2000) but, by comparison, the role of gene flow is less often considered. When it is considered, particularly in recent studies, gene flow has repeatedly been reported to play a critical role in adaptive divergence. This role can be both constraining and diversifying. The constraining effect comes when populations in different environments exchange genes with each other (e.g. King and Lawson, 1995; Hendry *et al.*, 2002; Calsbeek and Smith, 2003; Saint-Laurent *et al.*, 2003; Nosil and Crespi, 2004; Hendry and Taylor, 2004). The diversifying effect comes when populations in similar environments experience different levels of gene flow from a different environment (Garant *et al.*, 2005; Postma and van Noordwijk, 2005). These apparently dichotomous effects are really just two sides of the same coin, as illustrated in the present study. That is, gene flow from the lake constrained divergence of outlet fish from lake fish (a different environment) but caused divergence of outlet fish from inlet fish (a similar environment). In short, the observed amount of divergence among populations should not be used to infer local adaptation. To make such inferences, one should first quantify the interacting effects of gene flow and selection (Hendry and Taylor, 2004; Garant *et al.*, 2005; Postma and van Noordwijk, 2005). The present study demonstrates that it is possible to reveal the effects of gene flow by showing that morphological variation cannot be explained solely by selection.

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