

PHENOTYPE-DEPENDENT NATIVE HABITAT PREFERENCE FACILITATES DIVERGENCE BETWEEN PARAPATRIC LAKE AND STREAM STICKLEBACK

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Adaptive divergence between adjoining populations reflects a balance between the diversifying effect of divergent selection and the potentially homogenizing effect of gene flow. In most models of migration-selection balance, gene flow is assumed to reflect individuals' inherent capacity to disperse, without regard to the match between individuals' phenotypes and the available habitats. However, habitat preferences can reduce dispersal between contrasting habitats, thereby alleviating migration load and facilitating adaptive divergence. We tested whether habitat preferences contribute to adaptive divergence in a classic example of migration-selection balance: parapatric lake and stream populations of three-spine stickleback (*Gasterosteus aculeatus*). Using a mark-transplant-recapture experiment on morphologically divergent parapatric populations, we showed that 90% of lake and stream stickleback returned to their native habitat, reducing migration between habitats by 76%. Furthermore, we found that dispersal into a nonnative habitat was phenotype dependent. Stream fish moving into the lake were morphologically more lake-like than those returning to the stream (and the converse for lake fish entering the stream). The strong native habitat preference documented here increases the extent of adaptive divergence between populations two- to fivefold relative to expectations with random movement. These results illustrate the potential importance of adaptive habitat choice in driving parapatric divergence.

KEY WORDS: Adaptive divergence, dispersal, *Gasterosteus*, migration-selection balance, parapatry, reproductive isolation.

Local adaptation is important to the evolution of biodiversity, as it promotes ecologically significant phenotypic and genetic variation among habitats (Kawecki and Ebert 2004). When this variation influences patterns of mate choice, local adaptation may also lead to the evolution of assortative mating and perhaps speciation (Michalak et al. 2001; Hollander et al. 2005; Nosil 2007). Consequently, elucidating the mechanisms that promote or inhibit local adaptation can shed light on the origin of phenotypic diversity among populations, and ultimately ecological speciation.

Adaptive divergence is usually viewed as a balance between divergent selection and the constraining effect of gene flow (Slatkin 1985, 1987; Lenormand 2002). Although dispersal can be a source of genetic variation required for local adaptation (Garant et al. 2007; Kitano et al. 2008), numerous case studies indicate that high dispersal can constrain adaptive divergence among populations (Riechert 1993; Sandoval 1994; Ross and Keller 1995; Hendry and Taylor 2004; Nosil and Crespi 2004; Moore et al. 2007). These constraints can lead to local maladaptation (García-Ramos and Kirkpatrick 1997; Crespi 2000; Ronce and Kirkpatrick 2001; Bolnick and Nosil 2007; Bolnick et al. 2008). In turn, maladaptation can drive peripheral populations extinct and thereby establish species' range boundaries (Kawecki 1995; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997).

Most previous work on migration-selection balance has assumed that dispersal is a fixed parameter, whose value is independent of the strength of divergent selection or the phenotypes of potential dispersers (Haldane 1930; Bulmer 1971, 1972; Kawecki and Holt 2002; Lenormand 2002). This assumption can be violated by either of two conceptually distinct but not mutually exclusive forms of nonrandom dispersal: (1) native habitat preference, and (2) phenotype-dependent habitat preference.

Native habitat preference occurs when individuals select habitats that more closely resemble their native environment (Wecker 1963; Davis and Stamps 2004). For instance, in some species individuals imprint on visual, olfactory, or other cues during early development (Stamps and Davis 2006; Stamps and Blozis 2006; Selonen et al. 2007; Mabry and Stamps 2008). One consequence of native habitat preference is that the rate of migration between two contrasting environments will tend to be lower than expected based on individuals' capacity for movement. In addition to merely lowering the actual migration rate, native habitat preferences can lead to a correlation between migration rate and the strength of divergent selection. This correlation can arise because individuals might distinguish more readily among more divergent habitats when sampling prospective sites for settlement. Such a correlation departs from classic migration-selection models, where the migration rate is fixed and independent of the strength of divergent selection.

Phenotype-dependent habitat choice is less widely documented than native habitat preference. This form of nonrandom movement occurs when individuals maximize their fitness by opting to settle in whichever habitat is best suited to their particular phenotype (Rausher 1984; Jaenike and Holt 1991; Clobert et al. 2001; Edelaar et al. 2008). This phenomenon is also sometimes called "adaptive" or "matching" habitat choice (Edelaar et al. 2008). Phenotype-dependent habitat choice may be a mechanism for native habitat preference. An individual's natal habitat can induce plastic phenotypic changes that subsequently improve performance in, and thus create a preference for, the natal habitat. For instance, tiger snakes reared in arboreal, terrestrial, or aquatic habitats develop higher locomotor efficiency in their juvenile habitat, resulting in a corresponding native habitat preference (Aubret and Shine 2008). Note that this preference does not require any imprinting on the native habitat, but rather can be mediated via phenotypic traits and performance.

Individuals' performance in different habitats may also depend on heritable traits. In this case, phenotype-dependent habitat preference may establish a correlation between genotype and environment. For example, different genetic color morphs of the pygmy grasshopper *Tetrix undulata* select substrates and microclimates that optimize their body temperature and reduce predation risk (Ahnesjö and Forsman 2006). Such habitat preference may be matched by active avoidance of habitats in which an individual has low performance (Forbes et al. 2005). In effect, habitat use and ecological phenotype are pleiotropically linked (e.g., Via 1986; Hawthorne and Via 2001). Alternatively, phenotype-habitat correlations can arise via physical linkage between separate genes for phenotype and for preference. Finally, habitat preference may be under independent genetic control from phenotypic traits affecting performance, but can be brought into linkage disequilibrium with phenotypic traits via strong selection and/or habitat-based assortative mating.

Either form of habitat preference (native or phenotype-dependent) should facilitate evolutionary divergence. Native habitat preference reduces the effective migration rate, shifting the outcome of migration-selection balance toward greater adaptive divergence. Phenotype-dependent habitat preference changes the phenotypic and/or genetic identity of migrants, thereby reducing the input of maladapted individuals into a habitat (Armsworth and Roughgarden 2005, 2008). Indeed, strong phenotype-dependent migration can actually facilitate rather than oppose evolutionary divergence. Both native and phenotype-dependent habitat preferences can generate a positive feedback loop: moderate adaptive divergence can favor the evolution of additional habitat preference, which in turn promotes additional adaptive divergence (Rice and Hostert 1993; Ravigne et al. 2004). In extreme cases, this feedback might lead to the cessation of migration, habitat-based reproductive isolation, and hence parapatric or sympatric speciation

(Maynard Smith 1966; Rausher 1984; Bierne et al. 2003; Fry 2003; Tonnis et al. 2005; Korol et al. 2006). Such feedbacks are particularly relevant to ecological speciation in phytophagous insects, whose host plants serve as habitat, food, and mating sites (Dres and Mallet 2002; Emelianov et al. 2003; Linn et al. 2003). Habitat preferences thus can directly lead to speciation (Berlocher and Feder 2002).

STUDY SYSTEM

We tested whether habitat preferences contribute to parapatric divergence between adjoining lake and stream populations of three-spine stickleback (*Gasterosteus aculeatus*). Marine stickleback invaded freshwater habitats following Pleistocene deglaciation, and subsequently underwent adaptive divergence in sympatry within lakes (Ólafsdóttir et al. 2007), in parapatry between adjoining lake and stream habitats (Hendry et al. 2002), and in allopatry among watersheds (Lavin and McPhail 1986).

Parapatric populations of lake and stream stickleback have been a valuable system for studying the balance between dispersal and natural selection. Inlet and outlet streams of many lakes contain resident stickleback populations that are morphologically and genetically distinct from their neighboring lacustrine populations (Reimchen et al. 1985; Lavin and McPhail 1993; Thompson et al. 1997; Hendry and Taylor 2004; Moore and Hendry 2005; Moore et al. 2007). Stream stickleback have consistently deeper bodies and fewer, shorter gill rakers (relative to body size), which are thought to be adaptations to different hydrodynamic environments and prey availability (Berner et al. 2008). Defensive traits such as armor plate number and spine length also differ between lake/stream populations, although the direction of difference is not always consistent across independent population pairs. Common garden experiments have repeatedly confirmed that these morphological differences have a genetic basis (Hendry et al. 2002; Sharpe et al. 2008). Adaptive genetic divergence is further implicated by the recent finding that QTLs for body shape show exceptionally high F_{ST} between adjoining lake and stream pairs (relative to putatively neutral markers; R. Kaeuffer, A. Hendry, D. Bolnick, and C. Peichel, unpubl. ms).

The spatial scale of morphological divergence between lake and stream populations is remarkably small. In outlet streams, these morphological characters can show gradual or abrupt clines, changing from lacustrine to riverine forms over the space of hundreds or thousands of meters (Berner et al. 2008, 2009). Inlet streams tend to exhibit far more abrupt transitions between lake and stream phenotypes, often over a scale of a few meters (Moore and Hendry 2005). These small-scale clines have previously been interpreted as an outcome of strong divergent selection overcoming the homogenizing effects of gene flow (Moore et al. 2007).

Divergent selection is supported by reciprocal transplants showing that lake and stream fish grow better in their respec-

tive habitats (Hendry et al. 2002). In addition, a release–recapture experiment in a stream site produced higher recapture rates for stream natives than for lake fish, suggesting the possibility of viability selection against immigrants (Hendry et al. 2002). Despite this apparent divergent selection, morphological divergence between lake and stream populations is believed to be limited by gene flow (Berner et al. 2008). Outlet streams exhibit genetic clines: ongoing gene flow results in high genetic similarity between lake populations and nearby stream fish, but genetic similarity declines with distance from the lake (gradually or abruptly, depending on the population; Berner et al. 2009). Moore et al. (2007) estimated that gene flow from lakes reduces morphological divergence of outlet stream fish by > 80% relative to what might occur in the absence of gene flow.

Given that stickleback can travel up to 150 m over a few days (see Results), it seems unlikely that abrupt clines (sometimes within meters of the lake/stream transition) are maintained purely by selection against immigrants. Consequently, we hypothesized that lake and stream stickleback exercise strong habitat preferences that substantially reduce dispersal between lakes and streams, and may directly promote divergence. Using a mark-transplant-recapture experiment, we found evidence for phenotype-dependent and native habitat preferences in a divergent pair of lake and stream populations.

Methods

STUDY SITE

Blackwater Lake is a medium-sized (37.2 ha) mesotrophic lake in the Amor de Cosmos watershed, on northern Vancouver Island (50°10'40"N, 125°35' 20"W; see Caldera and Bolnick 2008 for a map of the watershed). Several lakes further up the watershed (Amor, Ormond, Surprise Lakes) drain into Blackwater Lake via a 1.2 km stream that drops ~ 25 m, mostly in the upper half of the connecting stream. The inlet stream has an appreciable current along almost all its length, even at the low-gradient end where it drains into Blackwater (0.135 to 0.503 m/s). There is a decayed beaver dam 70 m upstream, which creates a small cascade (0.25 m drop). Stickleback in Blackwater Lake are genetically divergent from upstream lakes, because the high-gradient upper end of the inlet stream lacks stickleback entirely (Fig. 1) and thus effectively prevents gene flow (Caldera and Bolnick 2008). Blackwater Lake exhibits no detectable genetic divergence from its downstream neighbor, Farewell Lake, due to the low-gradient connecting stream (1.2 km long, < 5 m drop) that contains stickleback throughout.

MARK-TRANSPLANT-RECAPTURE STUDY

On 10–11 June 2007, we captured 761 lake fish and 701 stream fish using unbaited minnow traps. The experiment occurred during

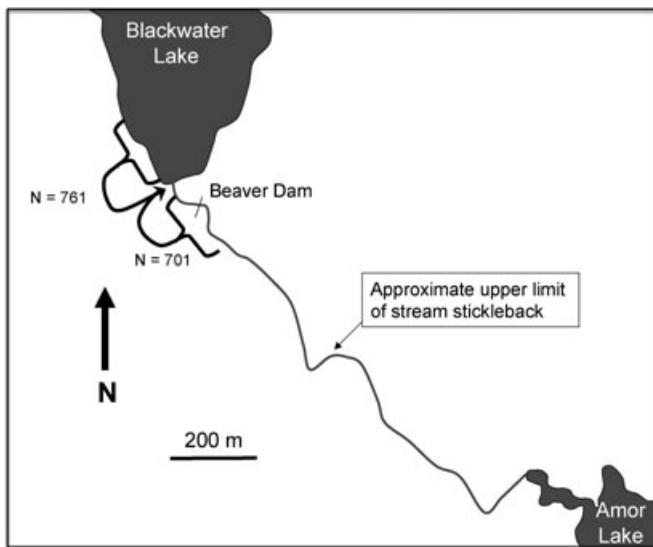


Figure 1. A map of the research site. The inlet stream drains from Amor Lake into Blackwater Lake (only part of each lake is drawn). A beaver dam approximately 70 m upstream from Blackwater presented a partial barrier to dispersal, and trapping found no detectable stickleback populations above 400 m from Blackwater lake. The diagram illustrates the experimental scheme, with 761 lake and 701 stream fish marked and transplanted to the stream mouth. The bracket along the lakeshore represents the scale of retrapping along the shore, whereas in the stream, retrapping extended 500 m upstream.

the extended breeding season for stickleback in this watershed. All fish were captured within 100 m of the mouth of the inlet stream. Fish were marked by subcutaneous injection of a small dot of fluorescent red (lake-caught fish) or yellow (stream-caught fish) elastomer dye (Northwest Marine Technologies, Shaw Island, WA). The dye spots were small, and only faintly visible without an ultraviolet light source, so were unlikely to significantly increase predation risk. All work was carried out with approval from the University of Texas Institutional Animal Care and Use Committee. Once fish had sufficient time to recover from handling (> 30 min), lake and stream fish were released in mixed batches of ~50. We released fish 2 m upstream from the lake, to ensure that the fish were in a channel that permits equal opportunity for movement up into the stream, and down into the lake. Releasing precisely at the mouth would have provided a 180° arc of potential movement into the lake, and a much smaller arc leading upstream.

Fish were recaptured four days later ($N = 252$ recaptures) using unbaited minnow traps. Traps were set at 5-m intervals from the mouth upstream to the beaver dam at 70 m, then 10-m intervals up to 150 m, and then 50-m intervals up to 500 m from the lake. No stickleback were detected more than 400 m from the lake. In the lake, 25 traps were set within 25 m of the inlet (near traps) and an equal number of traps were set between 25 and 100 m

from the inlet (far traps), concentrated along the shoreline where capture rates tend to be higher. For each location, we recorded the total number of stickleback captured, and the number of lake and stream recaptures. All recaptured fish were fixed in formalin, then rinsed and preserved in 70% isopropanol for morphometric analysis. In addition, we preserved 50 unmarked fish from the lake and up to 30 from each 10-m interval of the stream. We placed a fin clip from each specimen into ethanol for genetic analysis.

We distinguish between the rate at which individuals return to their original habitat (habitat fidelity), and the degree to which this return rate departs from a null expectation (habitat preference). Hence, habitat fidelity significantly greater than 0.5 implies native habitat preference. Given fidelity value f , the strength of preference is $c = 2(f - 0.5)$. We use “native habitat” to refer to the habitat in which a fish was originally captured, not necessarily where it was born or spent the majority of its life. We used a binomial test to evaluate whether lake (or stream) fish were recaptured in the lake (stream) more often than expected by chance (50% per habitat), and to obtain confidence intervals for our observed habitat fidelity.

In addition to testing whether fish returned to their native habitat more often than expected by chance, we examined the dispersal distances into each habitat. We first tested whether stream natives dispersed significantly less far into the lake than lake natives did, using a chi-square test contrasting lake versus stream recapture numbers in near-lake versus far-lake traps. Next, we tested whether lake natives dispersed significantly shorter distances upstream than stream natives did. For this test, we compared the median upstream distances for lake versus stream natives (conditional on their capture in the stream). The significance of this comparison was evaluated using a permutation test, randomizing native habitat across recapture distances and recalculating 10,000 null differences between lake and stream medians. Finally, we examined the effect of distance versus physical barriers (the beaver dam) in constraining upstream dispersal. Rather than assuming an a priori dispersal kernel (e.g., linear, Gaussian, or negative binomial; Coombs and Rodríguez 2007) we used a nonparametric generalized additive model (using the *gam* package in R; R Development Core Team 2007). This model tested for significant effects of distance upstream (smoothed) and location above/below the beaver dam (categorical) on the proportion of recaptured fish (out of all fish captured at each site), using binomial error terms. We used a log-likelihood ratio test to determine whether including the beaver dam (an abrupt break) significantly improved model fit over a smoothed nonlinear curve.

GENETIC DIVERGENCE

We used a Qiagen DNeasy tissue kit to extract DNA from fin clips of 49 unmarked lake individuals, and 67 unmarked individuals captured > 200 m upstream from the lake. Individuals

were genotyped for six microsatellites (stn130, stn171, stn184, stn195, stn207, and stn321) using reverse-labeled primers and PCR conditions described by Peichel et al. (2001), and run out on an ABI 3130 with GENESCAN ROX500 size standards. We used STRUCTURE (Pritchard 2000) to test whether the data supported the presence of two (lake/stream) or only one genetic population. Using ARLEQUIN (Schneider et al. 2000), we calculated Slatkin's linearized F_{ST} between lake and upper-stream fish, and tested for statistical significance using an AMOVA based on R_{ST} (overall, and for each locus separately). One of the loci (stn321) was selected because it exhibits exceptionally strong lake/stream divergence in five of six replicate lake/stream pairs in separate watersheds (high F_{ST} relative to putatively neutral markers; R. Kaeuffer, A. Hendry, D. Bolnick, and C. Peichel, unpubl. ms). This strong divergence likely represents the effect of hitchhiking from strong divergent selection on a nearby locus, as stn321 is linked to a QTL for body shape, found in an analysis of an F2 cross between freshwater and marine stickleback (Albert et al. 2008).

MORPHOMETRIC ANALYSIS

To confirm that morphological divergence was occurring between the lake and stream populations examined here, we measured body mass to 0.001 g on 43 unmarked lake fish, and 137 unmarked stream fish (~10 individuals from each of the following distances upstream from the mouth: 0, 10, 20, 30, 50, 70 [below dam], 80, 100, 120, 150, 200, 250, 300, and 350 m). We used digital calipers (accurate to 0.01 mm) to record standard length, body width (at pelvic spine joints), gape width, and first dorsal spine length. For each individual, we counted gill rakers on the first gill arch, measured the longest gill raker with an ocular micrometer, and determined sex by examining gonad morphology. All specimens, as well as recaptured fish, were photographed for geometric morphometrics.

We tested for significant morphological differences between lake and stream fish for each individual morphometric trait (log-transformed) using an analysis of covariance (ANCOVA) with population and sex as fixed effects, standard length as a covariate, and all interaction terms. We tested for overall morphometric differences using a multivariate analysis of covariance (MANCOVA) with all traits dependent on population, sex, standard length, and interaction terms. Focusing within the stream, we then tested for significant morphological differences between samples below and above the old beaver dam, using univariate ANCOVAs and a MANCOVA. Finally, we tested for clines from the lake into the stream using regression of principal component axes of morphology versus capture location (distance upstream from the inlet mouth). For these analyses, lake fish were assigned distances of -50 or -20 m from the inlet ("far" or "near" samples). We tested for discontinuities in the cline using split-line regression. To se-

lect the optimal break point for a split regression line, we iterated through all sample sites and for each site obtained the sums of squares for a split regression with a break at that site. We selected the break point with the lowest sum of squares, and then used a partial F -test to compare the split-line regression against a simpler model with a single line. If a split-line model was supported, we then ran separate regressions to test whether slopes were significant for each line above and below the break point. We also tested two a priori break points (the inlet mouth, and the dam) against simple linear regressions. These morphometric analyses were done using the R statistical language.

To obtain a metric representing lake versus stream phenotypes, we used the software tpsDIG2 (Rohlf 2007) to digitize 23 homologous landmarks (following Svanbäck and Bolnick 2007) on photographs of the right side of all specimens collected during the recapture round of trapping. We then calculated relative warps on all specimens using tpsRelw (Rohlf 2007), and used R to calculate a linear discriminant function separating lake and stream fish. Using the discriminant function to reassign individuals to lake or stream groups, we determined the rate of correct reassignment.

Using the discriminant function axis, we tested for phenotype-dependent habitat choice using a logistic regression model in which individuals' dispersal behavior (returned to native habitat = 0; dispersed to alternate habitat = 1) was a function of native habitat (lake or stream), morphology (discriminant function score), and a native habitat \times morphology interaction. Phenotype-dependent habitat preferences should result in a significant interaction term, as stream natives recaptured in the lake will exhibit a more lake-like morphology than those returning to the stream, whereas lake natives recaptured in the stream will be more stream-like than those recaptured in the lake. A habitat effect alone would represent asymmetric dispersal between habitats, and a morphology effect would suggest that certain phenotypes are inherently more likely to disperse without regard to their origin. In addition, we ran separate one-tailed t -tests for lake and stream natives evaluating whether dispersers resembled fish from the alternate habitat more than nondispersers did.

Results

HABITAT CHOICE

Stickleback exhibited strong habitat fidelity, with 88.1% of recaptured fish returning to their native habitat (95% CI: 83.3–91.7; 222 out of 252 recaptures; $P < 0.0001$; Fig. 2). Compared with a null expectation of 50% fidelity, this represents a 76.2% reduction in dispersal, indicating very strong native habitat preferences. The return rate was similar for males and females (86.0% and 90.2% respectively $\chi^2 = 1.15$, $P = 0.28$), suggesting that native habitat preference was not merely a result of males returning to nesting territory.

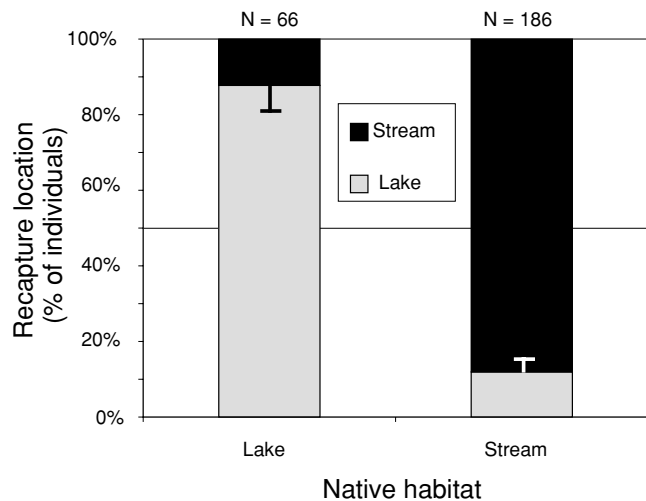


Figure 2. The relative frequencies with which lake and stream natives were recaptured in lake or stream habitats, four days after release. Standard error bars are provided based on a binomial distribution, and numbers above each bar represent the total number of lake and stream natives that were recaptured.

Of the fish that did move into a nonnative habitat, most did not venture far. In the lake, stream fish constituted almost half of the recaptures in the ‘near lake’ area within 25 m of the inlet mouth (20 out of 45 recaptures; null frequency = 0.479, $P = 0.748$), but were rarely captured further from the mouth (3 out of 35 recaptures further than 25 m from the mouth; null frequency = 0.479, $P = 7 \times 10^{-6}$). Hence, stream fish became proportionally rarer further from the stream mouth, relative to lake fish released at the same site ($\chi^2 = 10.68$; $P = 0.001$). Conversely, lake natives dispersed shorter distances upstream than stream natives did. The eight lake fish captured in the stream were an average of 21.3 m upstream (± 5.43 SE, max = 50 m, median = 20), as opposed to the mean of 44.1 m (± 2.99 SE; max = 150 m, median = 40) for stream fish returning to their native habitat (Fig. 3). A permutation test confirmed that the greater median distance observed in stream fish is statistically significant ($P = 0.047$).

Upstream movement of released fish was limited by both rate of dispersal (distance) and physical barriers (the decayed beaver dam 70 m upstream). The frequency of marked fish increased slightly moving upstream from the lake to the dam, declined abruptly at the dam, and then declined gradually thereafter (Fig. 3). Using a generalized additive model (gam) with both distance and dam effects, the fraction of marked fish caught at each site was a significant function of distance upstream ($\chi^2 = 8.55$; $df = 3$; $P = 0.036$). Removing the abrupt effect of the beaver dam significantly reduced model fit, judging by both Akaike information criterion (AIC) and a log-likelihood ratio test ($P = 0.0228$). Because the gam allows nonlinear curves, the abrupt drop in re-

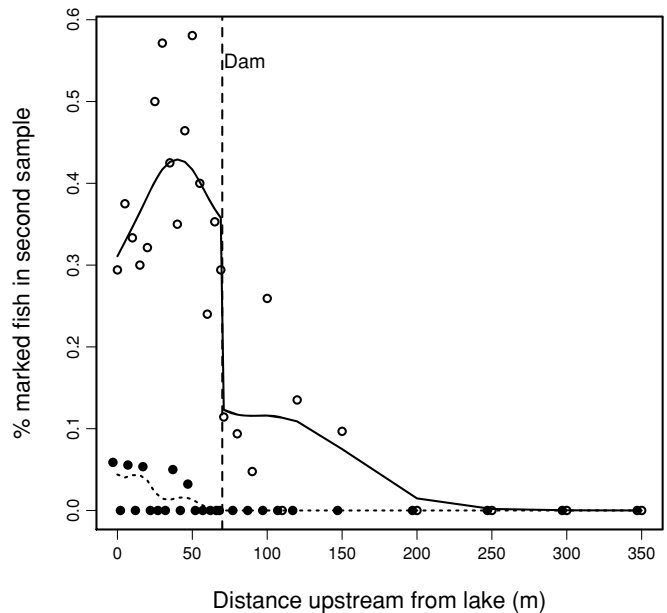


Figure 3. The extent of upstream dispersal by stream and lake natives (open and solid circles, respectively). The vertical axis is the fraction of recaptured stream (or lake) natives of the total number of fish captured at each site (mean $N = 24.7$ per 5-m increment, $SD = 10.7$). Curves are estimated by a generalized additive model with binomial error terms, and include distance as a continuous effect and above/below the dam as a categorical effect. The location of the dam is indicated by a vertical dashed line. Note the sharp drop in the frequency of marked stream fish, coinciding with the dam.

capture rates at the dam is not simply an artifact of a nonlinear dispersal kernel.

GENETIC DIVERGENCE

We found significant genetic differences between upper stream (200–350 m) and lake fish (Slatkin’s linearized $F_{ST} = 0.00846$, permutation-based P -value = 0.011; Fig. S1). An analysis of molecular variance (AMOVA) using R_{ST} found that between-population comparisons contributed only 0.86% of the overall genetic variation, but this was nevertheless statistically significant ($P = 0.008$). Looking at individual loci, two microsatellites (stn184 and stn171) approached statistical significance, and a third (stn321) exhibited significant among-population differences (Fig. S1). STRUCTURE found support for two genetic populations (likelihoods of $K = 1$ and $K = 2$ were -2251.8 and -2227.2 , respectively). However, when stn321 was excluded, $K = 1$ was supported instead.

MORPHOMETRIC ANALYSES

We found significant morphological differences between lake and stream populations (Pillai’s Trace = 0.392, $P < 0.0001$),

Table 1. Morphological differences between lake, lower stream, and upper stream samples, providing trait means and standard deviations (in parentheses). *P*-values are provided for population effects on each trait, from an ANCOVA with population, sex, and sex × population effects, with standard length as a covariate. Statistically significant contrasts are highlighted in bold. See Tables S1 and S2 for details of the ANCOVAs.

Trait	Lake	Stream below dam	Stream above dam	Lake vs stream	Above vs below dam
Sample size	43	76	61	<i>P</i> -value	<i>P</i> -value
Mass (g)	1.654 (0.763)	1.931 (0.792)	1.705 (0.699)	<0.001	<0.001
Standard length (mm)	48.827 (6.646)	49.984 (6.138)	48.160 (5.324)	0.668	0.076
Body width (mm)	6.050 (1.158)	6.715 (1.24)	6.282 (1.078)	<0.001	<0.001
Spine length (mm)	5.143 (0.605)	6.715 (0.595)	6.282 (0.607)	0.523	0.112
Gape width (mm)	5.143 (0.711)	5.137 (0.760)	5.280 (0.581)	0.001	<0.001
Gill raker number	20.628 (1.273)	20.724 (1.372)	20.705 (1.283)	0.712	0.953
Gill raker length (mm)	0.803 (0.116)	0.882 (0.123)	0.948 (0.125)	<0.001	<0.001

and between stream fish above versus below the beaver dam (Pillai's Trace = 0.400, $P < 0.0001$; see Table 1 for summaries, or Tables S1 and S2 for full ANCOVA and MANCOVA details). Adjusting for body size using a principal component analysis, stream fish tend to have more, longer gill rakers, although this effect is only significant above the beaver dam (Tables S3 and S4). Both lower and upper-stream fish have shorter dorsal spines, and shorter, deeper bodies than their lake counterparts (Tables S3 and S4).

There are significant linear clines as one moves upstream from the lake (Fig. S2). Of the seven principal component axes, only PC1 (size) and PC4 (spine length) fail to show significant linear clines ($P > 0.14$, all others $P < 0.01$; Table S5). For most traits, the data better fit discontinuous clines rather than simple linear trends (all PC axes $P < 0.05$ except for PC3 and PC7, which had $P < 0.1$). Optimal break points tend to fall close to or slightly below the beaver dam (40–60 m upstream; Table S5), except for one break at 15 m. Note that PC1 and PC4, which had no simple linear trend, both had significant trends once the break point was accounted for (Table S6).

PHENOTYPE-DEPENDENT HABITAT CHOICE

A discriminant function analysis of geometric relative warps was effective at distinguishing lake versus stream natives (Fig. 4): 86% of fish were correctly assigned to their native habitat. Using this discriminant function axis (DFA) as a measure of morphology, we found evidence for phenotype-dependent habitat preferences. Specifically, whether or not an individual dispersed into a nonnative habitat depended on an interaction between its morphology and its native habitat (logistic regression interaction term; $\chi^2 = 4.31$, $df = 1$, $P = 0.0358$). Neither the morphology nor habitat effects were significant alone ($P = 0.11$ and 0.089 , respectively), indicating that there was no tendency for a particular phenotype to be more likely to disperse in general, nor were natives of one habitat more likely to disperse. Looking more closely at the cause

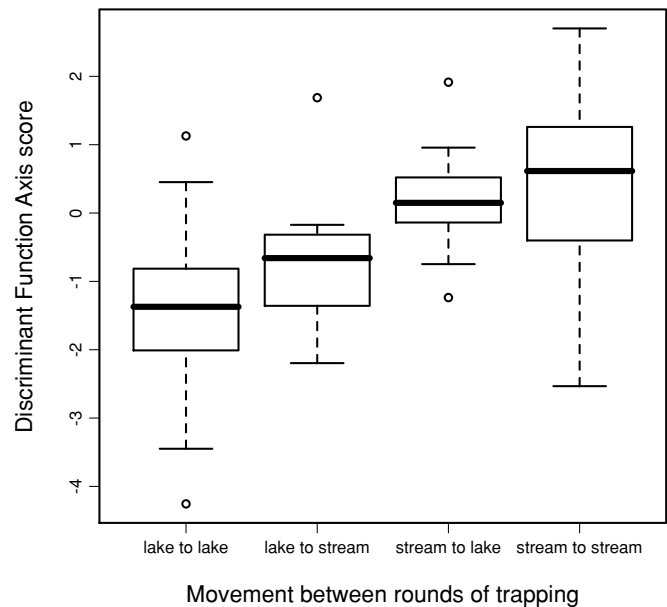


Figure 4. Boxplot of discriminant function axis scores from relative warps of geometric morphometric landmarks, for recaptured fish that returned to their native habitat (lake to lake, or stream to stream) or dispersed between habitats (lake to stream, or stream to lake).

of the interaction term (Fig. 4), we found that stream fish dispersing into the lake were more lake-like than stream fish that returned to their stream (mean DFA score for recaptures in the lake = 0.186, mean for recaptures in the stream = 0.458, $t = -1.618$, one-tailed $P = 0.055$). Conversely, lake fish dispersing into the stream were more stream-like than expected (mean DFA for recaptures in the stream = -0.647 , recaptures in the lake = -1.416 , $t = -1.76$, one-tailed $P = 0.055$). Although neither t -test is significant individually, a weighted Z test (Whitlock 2005) indicates it is unlikely to have two such nearly significant results co-occur by chance ($P = 0.050$), confirming that dispersing fish tend to resemble natives of their new habitat.

Given that we found evidence for phenotype-dependent habitat preference, we ran one additional set of analyses to determine the relative role of native habitat versus phenotype in determining individuals' recapture site. We used a logistic regression to test whether individuals' recapture site (scored as 1 [stream] or 0 [lake]) depends on native habitat and DFA score. Both factors were significant (native habitat: $\chi^2 = 34.71$, $P \leq 0.001$; DFA scores: $\chi^2 = 3.97$, $P = 0.0462$), but native habitat had a much stronger effect on final recapture site when both factors were included in a model simultaneously.

Discussion

Parapatric lake and stream populations of stickleback are a classic study system for migration-selection balance and adaptive divergence (Lavin and McPhail 1993; Hendry et al. 2001; Hendry and Taylor 2004; Moore et al. 2007; Berner et al. 2008). However, our results suggest that the degree of adaptive divergence is determined by more than simple migration-selection balance. Stickleback exhibited nearly 90% habitat fidelity, representing strong native habitat preference that reduced dispersal by 76% relative to null expectations. Given the short time frame of our recapture study (four days), it is possible that habitat fidelity would have been even larger if transplanted fish had more time to return to their native habitat. Supporting this suggestion, we found that fish that dispersed into a nonnative habitat tended to remain closer than expected to the release site. This low dispersal kernel increases the likelihood that the individuals might eventually return to their native habitat. Collectively, these results imply that stickleback exhibit native habitat preferences which may reduce dispersal between adjoining lake and stream habitats. In addition, we found evidence for phenotype-dependent habitat preferences.

Individuals that did disperse into a nonnative habitat tended to be morphologically more similar to natives of their new habitat than expected by chance. This suggests that stickleback exert some form of adaptive "matching" of their phenotype to their habitat (Edelaar et al. 2008). It is currently unknown whether this matching is a result of preferences for structural features of the habitat (depth, vegetation), preferences for different prey communities, or positive and negative rheotaxis (tendency to move up-current) on the part of stream and lake fish, respectively. It is also unknown whether the matching arises via induced preferences for natal habitat cues (Davis and Stamps 2004), or adaptive habitat choice based on performance differences arising from plastic or genetic morphological traits.

The short period between marking and recapturing fish makes it unlikely that phenotype-specific mortality explains the unequal recapture rates across populations. Although previous mark-recapture studies have found evidence for selection against immigrants between lake and stream habitats (Hendry et al. 2002),

this selection involved reduced growth rates over more than two weeks. Viability selection would have to be extraordinarily strong to explain the biased recapture rates in our experiment. Because we observe substantially overlapping phenotype distributions between lake and stream populations (Fig. S2), we consider such strong selection to be implausible.

Because our experiment was carried out in an inlet stream, it is possible that passive movement contributed to our results. If lake fish are less effective sustained swimmers, they may quickly be swept downstream back into the lake. However, this cannot be a complete explanation for our results, for three reasons. First, lake fish actually tend to be more effective sustained and burst swimmers (A. Hendry, pers. comm.), and so should be at least as capable of dispersing upstream. Second, if our results arose simply because lake fish were swept downstream, we would not expect to observe habitat choice by stream fish as well. In fact, stream fish showed just as strong native habitat preference, and those that did disperse into the lake remained disproportionately close to the stream inlet. Therefore, active habitat preferences must contribute to the patterns observed here (perhaps a combination of negative and positive rheotaxis by lake and stream fish, respectively). Third, phenotypic differences between dispersers and nondispersers (Fig. 4) indicate that habitat preference involves some degree of matching between individuals' morphology and habitat features.

Male stickleback are territorial and defend nests. Given that our experiment was conducted during the breeding season, it is conceivable that habitat preference was an inadvertent effect of fish returning to their home territories. We consider this explanation unlikely for two reasons. First, males and females exhibited similar levels of habitat preference, despite the fact that only the former are territorial. Second, more fish dispersed up past the beaver dam than were originally captured above the dam prior to marking and release, suggesting that individuals are not returning to their former territories. However, it is worth emphasizing that our measure of habitat preference is based on a single life-history stage (adults) at a single time of year. Ontogenetic and seasonal variation in habitat preferences may be a fruitful avenue for further work. In particular, it would be interesting to tease apart the roles of genetic divergence from induced habitat preferences.

MORPHOLOGICAL AND GENETIC DIVERGENCE

Like many prior studies of parapatric lake/stream divergence in stickleback (Reimchen et al. 1985; Lavin and McPhail 1993; Thompson et al. 1997; Hendry and Taylor 2004; Moore and Hendry 2005; Moore et al. 2007), we found significant morphological divergence between residents of the two habitats. Consistent with prior results, stream fish tend to be larger (marginally significant), have shorter dorsal spines, and deeper bodies (Tables S1 and S2; Fig. S2). In contrast to many lake/stream pairs (Berner

et al. 2008), gill raker length and number tended to be higher in the stream than in the lake. In other lake/stream pairs, such departures from the norm are associated with higher-than usual abundance of zooplankton in stream habitats (Berner et al. 2008), although we do not have data to evaluate this hypothesis in the present study. Another possibility is that high gill raker number and length result from the input of alleles from the upstream population in the large and deep Amor Lake, which has the longest and most numerous gill rakers in the entire watershed (D. I. Bolnick, unpubl. data).

Morphological divergence did not fit a simple linear cline (Fig. S2; Tables S5 and S6). Instead, there appeared to be abrupt changes (sometimes reversals) in slope for many traits. These transitions were almost all between the stream mouth and the beaver dam 70 m upstream. Roughly one-third of recaptured fish dispersed more than this distance within only four days. The fact that such significant morphological differences can be maintained despite this high capacity for movement, further supports our claim that habitat choice facilitates divergence. Without strong habitat choice, it is difficult to imagine such fine-scale clines could persist in the face of such high movement capacity.

Common-garden studies of other lake–stream pairs consistently find support for heritable trait differences (Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008). Surveys of putatively neutral genetic markers also consistently reveal significant genetic differences between lake and stream populations (Hendry 2004; Moore et al. 2007), particularly for inlet streams. Consequently, we expect there to be a genetic basis to the morphological cline between Blackwater lake and stream stickleback. Although our sampling scheme was comparable to these previous studies (sample size, number of markers, spatial scale), when examining five putatively neutral loci we found no significant allele frequency differences between populations (Fig. S1), although some markers approached significance. This represents a rare instance of an inlet stream population of stickleback that is only weakly distinct from the lake into which it drains; most inlet streams are strongly divergent even at neutral markers.

A sixth microsatellite marker (stn321, added subsequently) did show significant genetic differences between the lake and stream populations. This particular marker was specifically chosen because it is linked to a major QTL for body shape (roughly reflecting variation in body depth) in a study of crosses between a Japanese marine and Canadian freshwater (benthic) population (Albert et al. 2008). Body depth also differs consistently between lake and stream stickleback. Correspondingly, stn321 exhibits particularly high F_{ST} (relative to putatively neutral markers) in parallel across multiple lake/stream pairs in independent watersheds (R. Kaeuffer, A. Hendry, D. Bolnick, and C. Peichel, unpubl. data). We hypothesize that gene flow continues to be high enough (or genetic drift slow enough; or divergence recent enough) to homogenize most of the genome between the Black-

water lake/stream populations. Divergence may be constrained to parts of the genome (including stn 321) that are linked to loci either subject to strong divergent selection or contributing to genotype-dependent habitat choice.

THE ROLE OF HABITAT PREFERENCES IN ADAPTIVE DIVERGENCE

The strong native habitat preference documented here represents a substantial barrier to gene flow between lake and stream populations. Dispersal is reduced by at least 76% of what would be expected given the spatial proximity of these habitats and the substantial cruising range of stickleback. This reduced dispersal rate would greatly facilitate adaptive divergence between the populations in response to different selective pressures.

To illustrate the extent to which native habitat preference can increase evolutionary divergence, we turn to prior theoretical results describing migration–selection balance for a quantitative trait. Our goal here is to illustrate the potential amplifying effect of preference, rather than present a specific model of the lake/stream system. We focus on native habitat preference because it explained the majority of the variation in final recapture site. Hendry et al. (2001) showed that, when migration precedes selection within a generation, the equilibrium adaptive difference in a quantitative trait between populations is

$$D^* = \frac{\hat{D}}{m} \left[\frac{G}{G/m + (P - G + \omega^2)} \right], \quad (1)$$

where \hat{D} is the optimal trait difference, m is the sum of the migration rates in each direction, G is the genetic variance, P is the phenotypic variance, and ω^2 is the variance of the fitness function (inversely related to the strength of stabilizing selection). Extending these results, the impact of native habitat preference can be described by the ratio of adaptive divergence with versus without habitat preference:

$$\frac{D_c^*}{D^*} = \frac{G + m(P - G + \omega^2)}{G + m(1 - c)(P - G + \omega^2)}, \quad (2)$$

where c is the degree of native habitat preference (0 when individuals choose randomly, ranging up to 1 when individuals always return to their native habitat). Thus, the impact of native habitat preference is simply to reduce migration to a level below that which would be expected given individuals' locomotory ability. Note that empirical studies of migration rates using population genetics or mark–recapture methods estimate the actual rate of movement, $m(1 - c)$ and thus incorporate habitat preferences. The value of considering habitat preference in this model is to evaluate the extent to which active preferences (which may themselves evolve) can modify the outcome of migration–selection balance relative to a purely passive model of dispersal.

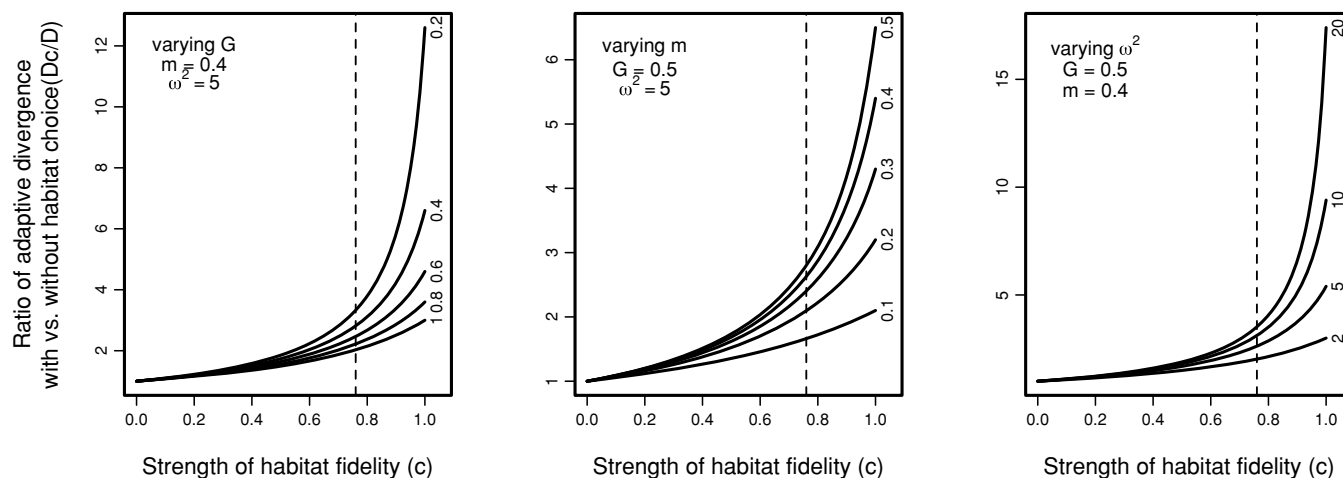


Figure 5. The effect of habitat fidelity (c) on adaptive divergence between populations. c ranges from 0, when individuals move randomly, up to 1 when preference for native habitat is complete. Dc/D is the ratio of adaptive divergence with versus without habitat choice (e.g., $Dc/D = 2$ implies that adaptive divergence is doubled by habitat choice). Separate curves present the effect of habitat choice (c) under different levels of heritability (G), random migration rate (m), and stabilizing selection (ω^2 is inversely related to strength of stabilizing selection). Vertical dashed lines indicate the empirically estimated value of c .

The effect of native habitat preference (c) on the degree of adaptive divergence is illustrated in Figure 5. We present the amplifying effect of habitat choice (eq. 3) while varying the genetic variance of the phenotypic trait (G ; equivalent to h^2 if we scale $P = 1$), the base migration rate in the absence of choice (m), and the strength of stabilizing selection (inversely related to ω^2). Numerical solutions show that the strong native habitat preferences documented here ($c \sim 0.762$) can increase adaptive divergence by between two- to fivefold (Fig. 5). Habitat preference facilitates adaptive divergence more when stabilizing selection is weak (large ω^2), dispersal is high (large m in the absence of habitat choice), or heritability is low (small G), each of which tends to inhibit adaptive divergence in traditional models of migration-selection balance.

To determine how much habitat preferences might facilitate divergence in stickleback specifically, we adopted the following parameter estimates. We scaled phenotypic variance to 1, and then used empirical measures of heritability to estimate a plausible value of $G = 0.31$, the median h^2 of 33 traits measured in a Californian stream population of stickleback (Baumgartner 1995). We assumed that, in the absence of habitat choice, migration over the few hundred meters studied here would be sufficient to fully mix the populations ($m = 0.5$). Finally, we assumed weak stabilizing selection $\omega^2 = 5$, implying that the stabilizing selection function has five times higher variance than the phenotype distribution. Given these assumptions, the native habitat preference documented here will lead to roughly 320% more adaptive divergence than would occur without habitat preference.

A few caveats are important in interpreting this model. First, this equation continues to assume that dispersing individuals are

randomly drawn from the source population's phenotype distribution. When individuals select a habitat that best matches their phenotype (as shown in our study), migration can actually facilitate adaptive divergence. However, at present we focus on native habitat preference for sake of illustration both because native habitat preference is the stronger force here, and because incorporating phenotype-dependence adds a layer of detail that requires more development than is feasible within this article. Armsworth and Roughgarden (2008) present a more extensive theoretical analysis of the effects of phenotype-dependent dispersal. Another caveat is that we assume selection is uniform within the two habitats. In reality, stream habitats in particular may vary substantially over a fine spatial scale (pool/riffle structure). There may thus be variation in selection intensity and direction within the stream. Furthermore, stickleback may show fine-grained habitat preferences even within the stream or lake habitat. Evidence for the latter form of habitat preference comes from recent studies of showing that, within panmictic lacustrine populations of stickleback, there is among-individual variation in diet associated with microhabitat use and trophic phenotype (Bolnick et al. 2003; Svanbäck and Bolnick 2007; Snowberg and Bolnick 2008; Araújo et al. 2008).

CONCLUSIONS

We found evidence for both native- and phenotype-dependent habitat preferences in threespine stickleback. Such preferences violate assumptions of most existing theory of migration/selection balance (but see Armsworth and Roughgarden 2008), but may be widespread (Edelaar et al. 2008). When such preferences occur, genetic and phenotypic divergence can be increased

severalfold. This increased divergence could in turn lead to stronger habitat preferences, resulting in a positive feedback loop driving increased habitat-based reproductive isolation (Rice and Hostert 1993; Hendry 2004; Tonnis et al. 2005). Consequently, our evidence for both native habitat preferences and phenotype-dependent habitat preferences suggests that dispersal behavior can play a crucial role in promoting parapatric diversification.

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Supporting Information

The following supporting information is available for this article:

Figure S1. Distribution of dinucleotide microsatellite alleles (PCR amplicon length) in lake versus stream populations for the five microsatellites examined here.

Figure S2. Morphological clines as a function of the distance in meters upstream from the inlet mouth.

Table S1. Analysis of covariance for individual morphometric traits comparing lake versus stream fish.

Table S2. Analysis of covariance for individual morphometric traits comparing stream fish from above versus below the dam.

Table S3. Principal component axis loadings for the seven morphometric variables in the study, with percent variance explained by each principal component axis, and a verbal interpretation of the meaning of each axis.

Table S4. Mean principal component axis loadings for lake and stream stickleback, distinguishing between above- and below-dam stream populations.

Table S5. Results from piecewise regression.

Table S6. Slopes of regression lines below and above the optimal break point, with standard errors of the slope estimates, t , and P -values.

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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