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ADAPTIVE DIVERGENCE AND THE BALANCE BETWEEN SELECTION AND GENE FLOW: LAKE AND STREAM STICKLEBACK IN THE MISTY SYSTEM

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Abstract.—We investigated the interplay between natural selection and gene flow in the adaptive divergence of threespine stickleback (*Gasterosteus aculeatus*) that reside parapatrically in lakes and streams. Within the Misty Lake system (Vancouver Island, British Columbia), stickleback from the inlet stream (flowing into the lake) have fewer gill rakers and deeper bodies than stickleback from the lake—differences thought to facilitate foraging (benthic macroinvertebrates in the stream vs. zooplankton in the open water of the lake). Common-garden experiments demonstrated that these differences have a genetic basis. Reciprocal transplant enclosure experiments showed that lake and inlet stickleback grow best in their home environments (although differences were subtle and often not significant). Release-recapture experiments in the inlet showed that lake fish are less well-suited than inlet fish for life in the stream (higher mortality or emigration in lake fish). Morphological divergence in the wild and under common rearing was greater between the lake and the inlet than between the lake and the outlet. Genetic divergence (mitochondrial DNA and microsatellites) was greatest between the lake and the upper inlet (1.8 km upstream from the lake), intermediate between the lake and the lower inlet (0.9 km upstream), and least between the lake and the outlet stream (1.2 km downstream). Relative levels of gene flow estimated from genetic data showed the inverse pattern. The negative association between morphological divergence and gene flow is consistent with the expectation that gene flow can constrain adaptation. Estimated absolute levels of gene flow also implied a constraint on adaptation in the outlet but not the inlet. Our results suggest that natural selection promotes the adaptive divergence of lake and stream stickleback, but that the magnitude of divergence can be constrained by gene flow.

Key words.—Adaptation, dispersal, *Gasterosteus aculeatus*, microsatellites, migration, mitochondrial DNA, natural selection.

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Theoretical work has suggested that adaptive divergence proceeds as a balance between the strength of diversifying natural selection and the amount of homogenizing gene flow (Haldane 1948; Slatkin 1973; Felsenstein 1976; Endler 1977; Barton and Gale 1993; García-Ramos and Kirkpatrick 1997). Countless empirical studies have confirmed the importance of natural selection in adaptive divergence (Endler 1986; Schluter 2000) but the relative importance of gene flow remains controversial (Ehrlich and Raven 1969; Slatkin 1987; Storfer 1999). It would be helpful to resolve this controversy because the extent to which gene flow constrains adaptive divergence has several important consequences. First, gene flow may keep populations from reaching local adaptive peaks (García-Ramos and Kirkpatrick 1997; Hendry et al. 2001), which can decrease mean population fitness and perhaps limit species' geographical ranges (Kirkpatrick and Barton 1997). Second, gene flow may prevent or delay the evolution of reproductive isolation, although natural selection can sometimes overpower this constraint (Rice and Hostert 1993; Gavrilets 2000; Schluter 2000). Third, gene flow may facilitate shifts between peaks on an adaptive landscape, thereby contributing to adaptive evolution (Peck et al. 1998). Finally, conservation strategies often propose the translocation of individuals among isolated populations (Griffith et al. 1989), which may depress the mean fitness of endangered populations by introducing maladaptive genes (Storfer 1999).

Quantifying the effects of gene flow on adaptive divergence has proceeded in two general spatial contexts: clines (including hybrid zones) and discrete populations. The theoretical and empirical work on clines has been much more detailed and comprehensive, particularly for Mendelian traits (Endler 1977; Barton and Gale 1993). However, many species are distributed among discrete habitat patches that are linked to varying degrees by gene flow, and many traits that reflect adaptation have a quantitative genetic basis. We therefore developed a theoretical context for quantitative traits in discrete populations (Hendry et al. 2001). In the present paper, we empirically investigate whether adaptive divergence represents a compromise between selection and gene flow for conspecific fish populations that reside in adjacent lakes and streams. Adaptive divergence between these environments has been documented for threespine stickleback (*Gasterosteus aculeatus*; Moodie 1972a,b; Reimchen et al. 1985; Lavin and McPhail 1993), juvenile coho salmon (*Oncorhynchus kisutch*; Swain and Holtby 1989), adult anadromous sockeye salmon (*Oncorhynchus nerka*; Hendry et al. 2000), and adult nonanadromous kokanee (*Oncorhynchus nerka*; Taylor et al. 1997). Lake and stream population pairs obviously experience divergent selection but, because of their proximity, they may also exchange many migrants (e.g., Hendry et al. 2000). Thus, it seems plausible that the adaptive divergence of some such pairs may be constrained by gene flow.

We chose threespine stickleback for exploring the lake-stream contrast because different freshwater stickleback populations show remarkably divergent adaptations (Schluter and McPhail 1992; McPhail 1994; Reimchen 1994), and yet divergence may be constrained by gene flow in certain locations (Bell and Richkind 1981; Bell 1982). Parapatric lake and stream stickleback have been described for three systems in British Columbia: Drizzle and Mayer Lakes on the Queen

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Charlotte Islands (Moodie 1972a,b; Reimchen et al. 1985) and Misty Lake on Vancouver Island (Lavin and McPhail 1993; Thompson et al. 1997). In each case, stream-dwelling fish have deeper bodies for their length (i.e., less streamlined) and fewer gill rakers. These differences appear to have evolved in response to their divergent foraging environments (McPhail 1994). In particular, streamlined bodies are better suited for sustained swimming, whereas robust bodies are better suited for burst swimming (Taylor and McPhail 1986), with sustained swimming presumably typical in lakes and burst swimming typical in streams. More gill rakers are better suited for feeding on planktonic prey and fewer gill rakers for feeding on benthic macro-invertebrates (Bentzen and McPhail 1984; Lavin and McPhail 1986), with zooplankton predominating in lakes and benthic macro-invertebrates predominating in streams (Hagen and Gilbertson 1972; Gross and Anderson 1984). Lake-stream pairs may also differ in a host of other traits: body size, coloration, lateral plate number, and pelvic spine length (Moodie 1972a; Reimchen et al. 1985; Lavin and McPhail 1993).

We conducted a detailed study of stickleback at different sites in the Misty Lake system (upper inlet, lower inlet, outlet, lake), as well as in an independent system for comparison (Mackie Outlet, Mackie Lake). First, we show that morphological divergence between stream and lake stickleback varies considerably within the Misty system (greatest for the upper and lower inlet, least for the outlet). Second, we use common-garden experiments with families from Misty Lower Inlet, Misty Outlet, and Misty Lake to demonstrate that the important morphological differences have a genetic basis. Third, we test for divergent adaptation using fish from Misty Lower Inlet and Misty Lake in reciprocal-transplant enclosure experiments (to monitor growth) and release-recapture experiments in the stream (to monitor survival and emigration). Fourth, we use mitochondrial DNA (mtDNA) and nuclear microsatellite DNA to show that, within the Misty system, the least morphologically divergent stream fish (outlet) are also the least genetically divergent, and thus experience the most gene flow from the lake. We discuss the morphological and experimental evidence that divergent lake and stream populations are adapted to their local environments. We then provide evidence that gene flow constrains adaptation in the least divergent stream population (outlet) but not in the most divergent stream population (inlet). We also consider possible alternative explanations for associations between morphological and genetic divergence.

Materials and Methods

Study Sites

Misty Lake is located approximately 15 km upstream of the ocean in the Keogh River system on northern Vancouver Island, British Columbia (Fig. 1; 50°36′32″N, 127°15′46″W). The lake has a surface area of 35.6 ha, a mean water depth of 1.7 m, and a maximum water depth of 6.1 m. The water is darkly stained with sphagnum moss tannins, and the littoral zone is covered during the summer with loose detritus and dense beds of *Potamogeton* and *Nuphar* (Lavin and McPhail 1993). Specific locations used for our study included Misty Lake (several locations along the lake margin), Misty Lower Inlet (0.9 km upstream of Misty Lake; 50°36′11.8″N, 127°14′58.1″W), Misty Upper Inlet (1.8 km upstream of Misty Lake; 50°35′46.4″N, 127°14′56.2″W), and Misty Outlet (1.2 km downstream of Misty Lake; 50°36′49.9″N, 127°16′26.0″W).

Mackie Lake is located approximately 11.1 km upstream of the ocean in the Pye Lake system on north-central Vancouver Island (Fig. 1; 50°15′33.6″N, 125°35′2.3″W). The lake has a surface area of 10.2 ha and a maximum water depth of at least 8 m (systematic soundings have not been made). The water is minimally stained, much of the bottom is covered with loose detritus up to 3 m deep, and aquatic macrophytes are few. The small inlet stream is steep and does not appear to contain stickleback. The outlet stream is partially isolated from the lake by a beaver dam, and then flows through 50 m of swampy area before coalescing into a relatively steep, fast-flowing stream. Specific locations used for our collections and experiments included Mackie Lake (several locations along the lake margin) and Mackie Outlet (40 m downstream of the lake).

Ideally, we would have collected data characterizing the strength of divergent selection for each lake-stream pairing. A formal analysis of selection using marked individuals was not feasible because reliable individual marks are not available for stickleback, and because accurate characterization of selection requires very large, replicated experiments (Kingsolver et al. 2001). An alternative approach is to quantitatively measure environmental variables expected to influence the evolution of stickleback morphology. We intend to collect such data, but have not yet done so. Thus, we here offer our qualitative assessment of the relative strengths of divergent selection in the Misty system. Misty Upper Inlet is undoubtedly the most divergent environment because it is the smallest and farthest upstream of the lake. Misty Lower Inlet is quite similar in character to Misty Upper Inlet, being only somewhat larger. Misty Outlet retains much of the same character as the inlet sites but may have slightly more lakelike features because it is larger and downstream of the lake. However, the environment of Misty Outlet is still much closer to that of Misty Inlet than to that of Misty Lake.

Morphological Analysis

We captured stickleback using pole seines and minnow traps. All fish were preserved in 95% ethanol to facilitate rapid collection, as well as subsequent morphological and genetic analyses. Several months after collection, we measured body length (tip of upper jaw to end of hypural plate), body depth (anterior insertion 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 left maxilla), number of gill rakers (left side of outermost gill arch), number of lateral plates (left side), and pelvic girdle width (at its widest). For each collection of fish in 1997–1999 (site- and year-specific), we measured all individuals (when less than 30 were collected) or 30 haphazardly selected individuals (when more than 30 were collected). Our measurements are not directly comparable to those in previous studies that used formalin instead of ethanol (e.g., Lavin and McPhail 1993).

Body length and traits not correlated with body length (gill

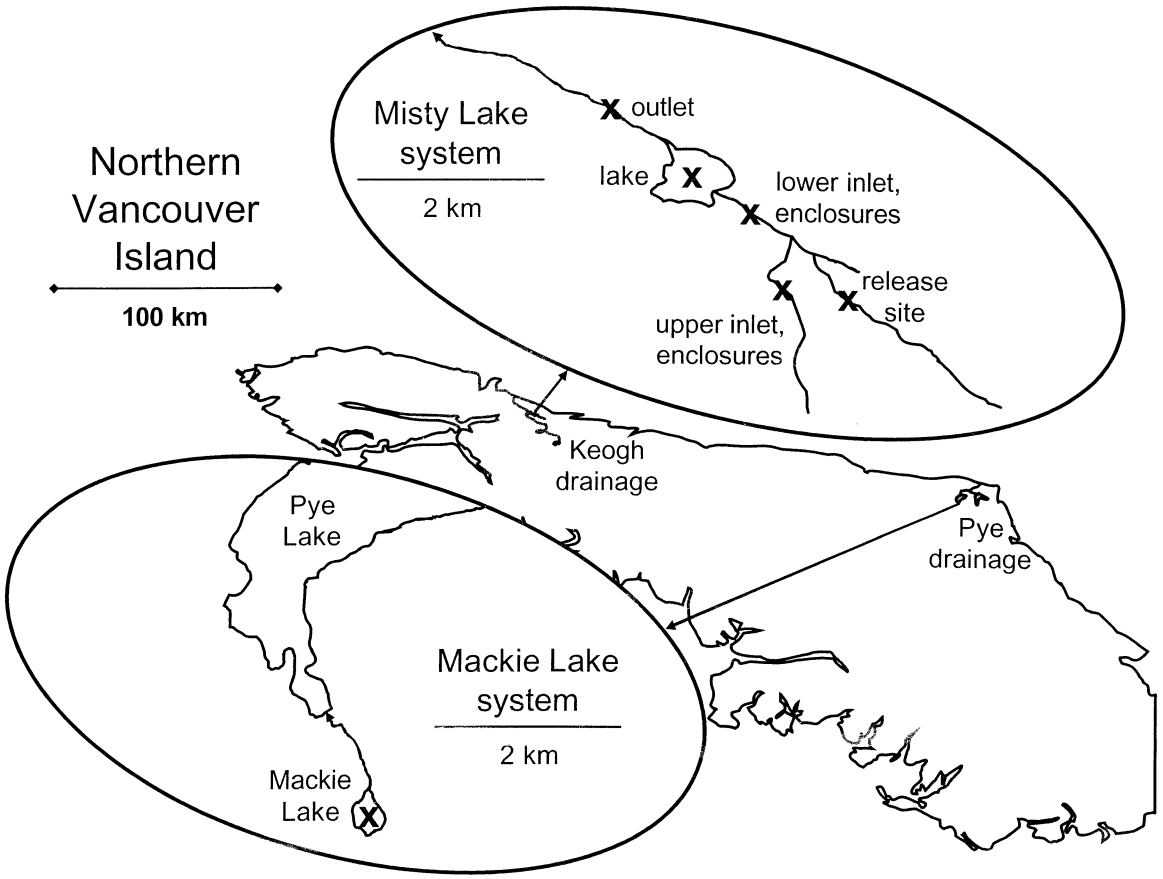

FIG. 1. A map of northern Vancouver Island, omitting all drainages except for Keogh and Pye. Insets show portions of the Keogh and Pye drainages that include Misty Lake and Mackie Lake, respectively. Arrows indicate the direction of stream flow. For the Misty system, crosses show the locations of sites where stickleback were collected for morphological and genetic analyses (lake, outlet, lower inlet, upper inlet), where stream enclosures were placed (12 in the lower inlet, 12 in the upper inlet), and where the release-recapture experiment was performed (release site).

rakers, lateral plates) were compared among collections using one-way ANOVAs and posthoc Tukey tests. Traits correlated with body length (body depth, pelvic spine length, upper jaw length, pelvic girdle width) were compared among collections using ANCOVA (all traits $\log_{10}$ transformed). The ANCOVAs first used a full model (with interaction) to test for heterogeneity of slopes. The interaction term was then removed (even when significant, because any heterogeneity was typically caused by a single collection) to test for variation among collections at a common body length (i.e., comparison of adjusted means). Allometric adjustments were then used to standardize trait sizes to the overall mean body length (55.4 mm), using $M_{std} = M_o(55.4/L_o)^b$, where M is trait size, L is body length, b is the ANCOVA slope with the interaction removed, and the subscripts *std* and *o* refer to standardized and observed measurements. Standardized trait sizes were compared among collections using one-way ANOVAs and Tukey tests. Discriminant functions were used to determine how well fish from each collection could be classified back to that collection based on morphology (four of the traits standardized as above).

Two types of Euclidean distance were used to quantify the total amount of morphological divergence among collections. For each collection, we first calculated mean values for each trait (four of the traits standardized as above) and converted these to *Z*-scores (i.e., mean = 0 and SD = 1 across collections). All pairwise Euclidean distances were then calculated using these *Z*-scores (D_z), which weighted each trait equally but did not account for correlations among traits. We next calculated mean values for each collection for each of the discriminant functions and used these to calculate all pairwise Euclidean distances (D_{cd}). Because this second method was based on discriminant functions, it weighted each of the original traits equally *and* accounted for correlations among traits. Average morphological divergence between sites was determined by averaging pairwise comparisons from different years (D_z and D_{cd} separately).

Common-Garden Experiment

We used minnow traps and seine nets to collect gravid females and mature males on 29 May 1999 from three locations (Misty Lower Inlet, Misty Outlet, Misty Lake). These fish were used to produce three full-sibling families for each of six cross types (inlet × inlet, inlet × outlet, inlet × lake, outlet × outlet, outlet × lake, lake × lake). Both types of maternal parent were used for each hybrid cross, thereby reducing the potential influence of population-specific ma-

ternal effects on variation among crosses. However, too few families were available to provide a formal test for such effects. The three fertilized egg masses for each cross type were pooled (owing to space limitations) and maintained in 100-L aquaria (one per cross type) using standard protocols (e.g., Hatfield and Schluter 1999). Hatching took place on 7–12 June 1999, and cross types with abundant progeny were later divided among additional aquaria (as aquaria became available). Food sources were *Artemia* nauplii, frozen bloodworms (*Chironomid* sp.), and frozen adult brine shrimp (*Artemia* sp.). On 11 June 2000, all fish were preserved in 95% ethanol. Several months later, up to 30 fish per cross type were measured and analyzed as described above for wild-caught fish (excluding discriminant functions and Euclidean distances). The overall mean length for allometric adjustments was 42.1 mm.

This common-garden experiment was designed to determine if the phenotypic differences observed in the wild had a genetic basis (variation among pure crosses should show the same pattern as that among wild fish) and if additive genetic variance made a substantial contribution to the differences (hybrid crosses should be intermediate to pure crosses). This design was not intended (and is not sufficient) to determine the genetic architecture of the traits or the relative contributions of maternal effects, additive genetic variation, and various types of nonadditive genetic variation. Such an analysis would be useful but requires a different design and more families, for which the necessary resources were not available.

Reciprocal Transplant Enclosure Experiments

We performed these experiments twice in Misty Lake (May 1999, June 2000) and once in Mackie Lake (July 1999), each time using enclosures originally developed for experiments on benthic and limnetic stickleback (Schluter 1995; Hatfield and Schluter 1999; Rundle 2002). For each experiment, we placed 24 enclosures in the open water of the lake (open-water enclosures), 24 along the shore of the lake (littoral enclosures), and 24 in the inlet or outlet stream (stream enclosures). The open-water enclosures were cylindrical columns of mesh (1 m in diameter), with a closed bottom and metal rings for support. They were extended to their full depth of 6 m in Mackie Lake but to only 4.5 m in Misty Lake (maximum lake depth is only 6.1 m). The enclosures were suspended from rafts made of 2 × 4s and styrofoam blocks, with the rafts anchored by ropes to cinder blocks on the lake bottom. The littoral enclosures were square (1 × 1 m) and 1.5 m in height, with an open bottom. They were placed in water approximately 1 m deep along the lake margin, and their bottom edges were embedded in the substrate to prevent fish from escaping. We used both open-water and littoral enclosures in lakes because lake fish might use both environments. The stream enclosures were identical to the littoral enclosures, and were placed in water 0.5–1.0 m deep where the current was slow (stickleback are commonly found in such areas). In Misty Lake, stream enclosures were placed in the inlet: 0.9 km above the lake (12 enclosures) and 1.8 km above the lake (12 enclosures). In Mackie Lake, stream enclosures were placed in the outlet, 40 m below the lake. These locations were chosen because they were the most suitable for placing the enclosures. Minnow traps were used to remove any wild stickleback from the newly placed littoral and stream enclosures.

Stickleback used in the experiments were captured from streams or along lake margins using unbaited minnow traps. Misty stream fish were captured from the location where the lower 12 enclosures were placed (lower inlet) and Mackie stream fish were captured from the location where all the enclosures were placed (outlet). Captured fish were held in coolers and processed within several hours. Processing involved selecting size-matched pairs (one lake, one stream) to be placed into each enclosure (gravid females and mature males were excluded). We used pairs rather than single fish to increase sample sizes and yet maintain independence among fish of each type. Each fish was clipped to provide an unambiguous mark (half of the right pelvic spine for stream fish and half of the left pelvic spine for lake fish) and then weighed in water to the nearest 0.01 g. The pairs of fish were held together in jars and then released into the enclosures within 6 h of their initial removal from the traps.

The fish were left undisturbed for 15–18 days, consistent with previous work (Schluter 1995; Hatfield and Schluter 1999; Rundle 2002). At the end of this interval, they were captured using minnow traps and aquarium nets (littoral and stream) or by removing the enclosures (open-water). Captured fish were held live in jars and then weighed to the nearest 0.01 g (as at the start of the experiment). Relative growth was calculated for each fish as the change in its mass divided by its initial mass, divided by the length of time. Although experimental fish varied in average size among pairs (e.g., Misty Lake 2000, CV = 34.6%), fish were size-matched well within pairs (e.g., Misty Lake 2000, stream mean = 1.76 g, lake mean 1.78 g, paired $t = 0.19$, $P = 0.189$, average difference within pairs = 6.6%). Because statistical tests were based on pairs, variation in initial mass would thus not influence differences between lake and stream fish.

These experiments are suitable for addressing two questions. First, within each type of enclosure in each experiment, do home fish (lake fish in the open water and littoral enclosures, stream fish in the stream enclosures) grow better (gain more mass or lose less mass) than foreign fish (stream fish in the open water and littoral enclosures, lake fish in the stream enclosures). This question was addressed using paired *t*-tests comparing the relative growth of lake and stream fish within each of the nine sets of enclosures (three enclosure types, three experiments). Second, do stream fish grow better in their home environment (stream enclosures) than in foreign environments (open-water and littoral enclosures) and vice versa for lake fish. If so, mean growth for each fish type should be higher in its home environment than in foreign environments. This question was addressed by calculating the difference in relative growth between lake and stream fish within each enclosure, and then comparing these differences among enclosure types using one-way ANOVA for each experiment.

Release-Recapture Experiments

We performed two release-recapture experiments (May 1999, June 2000), each conducted approximately 1.5 km up-

stream of Misty Lake in a tributary to the inlet (Fig. 1; 50°35′42.2″N, 127°14′4.0″W). The experimental section was 16.6 m long, 2–3 m wide, and 0.5–1.0 m deep, and was bounded by a series of shallow riffles (upstream) and a small dam of sticks, mud, and netting (downstream). Thus, it was possible, but difficult, for stickleback to leave the section. Downstream of the section, the stream was unsuitable for stickleback for approximately 250 m (shallow riffles with only a few small pools). In each year, we used unbaited minnow traps and seine nets to capture stickleback from Misty Lake and Misty Lower Inlet. The fish were held in coolers, from which we haphazardly selected equal numbers of lake and stream fish (excluding gravid females, mature males, and lake fish over 4 g). Half of a pelvic spine was clipped on all fish (left side for lake, right side for stream), and then they were released into the section within 6 h of their initial capture (136 stream and 136 lake fish on 16–17 May 1999; 135 stream and 134 lake fish on 17–18 June 2000). The section was then left undisturbed for two weeks (until 31 May 1999 and 1–3 July 2000), after which we used unbaited minnow traps and seine nets to recapture fish from the release site, as well as 300 m downstream and 100 m upstream. We recorded the identity (lake or stream) and capture location of each fish.

Before starting the 1999 experiment, we seined the release site and did not catch any stickleback. Before starting the 2000 experiment, we seined a few times and caught several stickleback (18 small fish without clips, one large fish with a clip). The site was therefore suitable for stickleback and yet none was present prior to our 1999 experiment, probably owing to the inhospitable section immediately downstream. The capture of a clipped fish before the 2000 experiment indicated that some fish released in 1999 had avoided capture and survived until the 2000 experiment. During sampling for the 2000 experiment, we therefore discriminated between fish released in 1999 and fish released in 2000 by checking for regeneration of the clipped spine (year-old clips regenerate to a short but sharp point, in contrast to the blunt point of newly clipped fish).

While seining at the end of the 2000 experiment, we caught 31 fish in the experimental section that had not been clipped. These fish were all small and fell into size classes suggesting they were progeny of fish released in 1999. Thus, some fish released in 1999 had produced offspring during that experiment or had avoided capture and bred later in 1999 or in 2000. We determined whether the unclipped fish were produced by lake mothers or stream mothers by screening them for the presence of two mtDNA clades that differ dramatically in frequency between the lake and inlet populations (see below). If lake mothers had produced the unclipped offspring, their clade frequencies would be shifted toward the frequencies in Misty Lake relative to those in Misty Lower Inlet (where the experimental stream fish were captured). This method could not determine how many mothers produced the offspring, nor could it ensure that the mothers had been a part of the release experiment (although this seems most likely).

Molecular Analyses and Population Genetics

We analyzed genetic variation among collections at one mtDNA restriction site and five nuclear microsatellite loci. Stickleback collected using seines and minnow traps in 1993–1996 were used for mtDNA analyses: Misty Lake (1993, 1995), Misty Lower Inlet (1993, 1994, 1996), and Misty Upper Inlet (1996). Stickleback collected in 1997–1999 and used for morphological analyses (see above) were also used for mtDNA and microsatellite analyses. Exceptions included Misty Upper Inlet 1997 (no microsatellite data), Mackie Lake 1998 (no mtDNA or microsatellite data), and Misty Outlet 1999 (no mtDNA or morphological data). The tail was removed from each preserved fish, and used to extract genomic DNA with standard proteinase K digestion using Gentra Systems (Minneapolis, MN) DNA isolations kits.

We first screened for the presence of two major ancestral mtDNA lineages (clades). Previous studies of the cytochrome *b* region of threespine stickleback mtDNA had revealed two major clades in the North Pacific: the Japanese clade, also called the Trans-North Pacific (TNP) clade, and the Euro-North American (ENA) clade. These clades differ by about 2.5% sequence divergence and can be diagnosed by the presence (TNP) or absence (ENA) of a single *Nsi* I restriction site (O'Reilly et al. 1993; Orti et al. 1994). Thompson et al. (1997) found both clades in the Misty system, and confirmed the utility of the *Nsi* I diagnostic test. We screened for these clades using restriction enzyme analysis of a portion of the cytochrome *b* gene amplified using the polymerase chain reaction (PCR). We used the primers GluDG and Cytb-2 (described in Palumbi 1996) in 25 μl reactions that included 1× PCR buffer (Bethesda Research Labs, Burlington, ON, Canada), 0.6 μM of each primer, 800 mM total dNTPs, 1.0 U of *Taq* polymerase (Bethesda Research Labs), and 2.0 mM $MgCl_2$. Reactions were processed in an MJ Research (Watertown, MA) PJC-100 thermal cycler: 1 cycle of 95°C denaturation (3 min), 52°C annealing (1 min), and 72°C extension (1 min); and 30–35 cycles of 92°C denaturation (30 sec), 55°C annealing (30 sec), and 72°C extension (30 sec). A final extension took place at 72°C (10 min). PCR products (approximately 500 base pairs) were incubated overnight with 10 U of *Nsi* I at 37°C, and the products were visualized under ultraviolet light on 2.5% agarose gels stained with ethidium bromide or SyberGreen (Molecular Probes, Inc., Eugene, OR). We tested for differences in clade frequencies among the collections using Monte Carlo chi-square permutations (Roff and Bentzen 1989) as implemented in Monte of the REAP software package (McElroy et al. 1992).

We next screened our 1997–1999 collections for allelic variation at five microsatellite loci. The loci had been isolated from stickleback genomic libraries and were assayed using PCR and radiolabelled primers as described by Rico et al. (1993, one locus) and Taylor (1998, four loci). Screening and genotyping procedures are detailed in Taylor (1998). GENEPOP 3.1c (Raymond and Rousset 1995) was used to test for deviations from Hardy-Weinberg equilibrium, linkage disequilibrium, and allelic frequency differences among all pairs of collections (genic differentiation). Permutation analyses in FSTAT 2.8 (Goudet 1995) were used to test whether each pairwise $F_{ST}(\theta)$ differed significantly from zero. Similarity of stickleback from the different collections (within the Misty and Mackie systems separately) was further assessed using assignment tests, which use multilocus genotypes to classify individuals to known collections characterized at those loci.

For this analysis, we used maximum-likelihood and jackknifing as implemented in GENECLASS (Cornuet et al. 1999). Genetic distances between collections were calculated using a variety of mutation-based and drift-based algorithms, all of which yielded similar results. Because drift-based methods are the most appropriate for postglacial freshwater stickleback (see Taylor and McPhail 2000), we report only Reynolds et al.'s (1983) coancestry coefficients.

Gene Flow and Its Effects

We estimated gene flow between Misty Lake and each of the three Misty stream sites (upper inlet, lower inlet, outlet). We first estimated the effective number of migrants (N_em) for each pair. N_em_{Wright} was calculated from the F_{ST}-values in Table 8 using Wright's infinite island model ($F_{ST} = 1/[1 + 4N_em]$). $N_em_{Takahata}$ was calculated from the same F_{ST}-values assuming two populations ($L = 2$) in Takahata's (1983) finite island model ($F_{ST} = 1/[1 + 4N_em(L/[L - 1])^2]$). $N_em_{Slatkin}$ was calculated using Slatkin's (1985) private alleles method as implemented in GENEPOP. N_em_{Beerli} was calculated using Beerli and Felsenstein's (1999) maximum-likelihood framework based on coalescent theory as implemented in MIGRATE. This program estimates the number of migrants into each population, which we summed to estimate total N_em. The above estimates thus represent the average number of effective migrants into each population (first three methods) or the total number of effective migrants between populations (N_em_{Beerli}). In each method, we first estimated N_em for all possible pairs of collections (based on different years) and then averaged those values for each lake-stream pairing. We estimated N_em using all four methods to allow comparison with other studies, but we place the most faith in the N_em_{Beerli} estimates because that method is tailored to pairs of populations and makes the fewest unrealistic assumptions (discussion of assumptions, Slatkin and Barton 1989; Beerli and Felsenstein 1999; Whitlock and McCauley 1999).

Hendry et al. (2001) showed that adaptive divergence is constrained by the rate of migration (m) rather than the number of migrants (N_em). Another benefit of the Beerli and Felsenstein (1999) method is that it allows simultaneous estimation of N_e and m for each population. We therefore estimated the rate of gene flow (m_{Beerli}) between each population pair using MIGRATE (assuming a mutation rate of $\mu = 10^{-4}$, Feldman et al. 1999). For comparison, we also estimated m by dividing N_em estimates from the other methods by a separate estimate of average N_e in each collection. These N_e estimates were calculated as $H/(1 - H)4\mu$ (Waples 1991), where H is the average expected heterozygosity (see Table 7) and μ is assumed to be 10^{-4}. We did not estimate N_e using linkage disequilibrium or temporal variation in allelic frequencies because our sample sizes were below the recommended minimum, our populations were large, and our samples spanned too few generations (see Waples 1991).

We next estimated the amount by which adaptive divergence between Misty Lake and Misty Outlet (where m was highest) might be constrained by gene flow. This analysis required a number of assumptions and the estimation of several imprecise parameters. The result is thus best viewed as heuristic (i.e., Could the estimated amount of gene flow have an important effect on adaptation under the specified conditions?). Our analysis was based on equation (7) in Hendry et al. (2001): $D^* = (D_\theta G)/(G[1 - \hat{m}] + [\omega^2 + P]\hat{m})$, where D^* is the equilibrium difference between populations in adaptive traits (assumed for simplicity to be the observed difference for the first canonical discriminant function), D_θ is the optimal difference (the parameter to be estimated), P is the phenotypic variance (assumed to be the average of the two sites = 0.74), G is the additive genetic variance (assumed to be $0.3P$, the median value for 33 stickleback morphometric traits in Baumgartner 1995), $\hat{m}$ is the proportion of individuals exchanged between sites (assumed to be $m_{Beerli} = 0.00267$), and ω is the strength of stabilizing selection within populations (i.e., width of the individual fitness function). Estimates of ω are not available for any stickleback populations and so we used an indirect approach. Turelli (1984) suggested that $1 + \omega^2/E = 20$, where E is the environmental variance. If we assume that $E = 0.43P$ (the median value from Baumgartner 1995), then $\omega^2 = 8.17P$. To a reasonable approximation, $\gamma = -1/\omega^2$, where γ is the quadratic selection gradient (Arnold et al. 2001). Turelli's (1984) estimate for ω^2 thus equates to $\gamma = -0.12P$, a strength of stabilizing selection commonly observed in natural populations (Kingsolver et al. 2001). We therefore used three different ω^2-values that, based on Kingsolver et al.'s (2001) review, would correspond to strong ($\omega^2 = 4P$, $\gamma = -0.25P$), moderate ($\omega^2 = 36P$, $\gamma = -0.03P$), and weak ($\omega^2 = 100P$, $\gamma = -0.01P$) stabilizing selection in nature. Using the above parameters in Hendry et al.'s (2001) equation, we estimated D_θ and then D^*/D_θ, the latter representing the degree to which divergence would be constrained by gene flow.

RESULTS

Morphological Variation in the Wild

Lake, inlet, and outlet stickleback differed in most morphological traits. Body length varied among collections ($F = 33.33$, $P < 0.001$), with lake fish usually being longer than stream fish, except for Misty Outlet (Table 1). The number of gill rakers varied among collections ($F = 22.6$, $P < 0.001$), with Misty Lake fish having more than Misty Inlet or Misty Outlet fish (Table 1; Fig. 2). The number of lateral plates also varied among collections ($F = 3.89$, $P < 0.001$), with Misty Inlet fish usually having more than Misty Lake or Misty Outlet fish (Table 1). Body depth was positively correlated with body length ($F = 942.32$, $P < 0.001$), and slopes were homogeneous among collections ($F = 0.62$, $P = 0.78$). Applying the allometric coefficient of $b = 1.061$, standardized body depth varied among collections ($F = 114.94$, $P < 0.001$), with Misty Inlet fish usually having deeper bodies than Misty Lake or Misty Outlet fish (Table 1; Fig. 2). Pelvic spine length was positively correlated with body length ($F = 171.49$, $P < 0.001$) and slopes were heterogeneous ($F = 3.23$, $P = 0.001$). Applying $b = 0.758$, standardized spine length varied among collections ($F = 9.58$, $P < 0.001$), with Misty Inlet fish usually having shorter spines than Misty Lake or Misty Outlet fish (Table 1). Upper jaw length was positively correlated with body length ($F = 318.92$, $P < 0.001$) and slopes were heterogeneous ($F = 2.07$, $P = 0.033$). Ap-

TABLE 1. Average morphological measurements of threespine stickleback from different collections in the Misty and Mackie systems. Body depth, pelvic spine length, upper jaw length, and pelvic girdle width were standardized to a common body length of 55.4 mm. Homogenous subsets of collections based on Tukey tests are indicated with letter superscripts. Collection sites include Misty Upper Inlet (MUI), Misty Lower Inlet (MLI), Misty Outlet (MO), Misty Lake (ML), Mackie Lake (MaL), and Mackie Outlet (MaO). Collection years include 1997 (97), 1998 (98), and 1999 (99).

	MUI97	MUI99	MLI97	MLI98	ML97	ML98	MO98	MaL98	MaL99	MaO99
N	7	30	21	30	30	30	29	30	30	30
Body length	$51.6^{b,c}$	49.1^{b}	36.2^{a}	$52.3^{b,c}$	62.8^{d}	61.6^{d}	$58.1^{c,d}$	63.5^{d}	61.2^{d}	49.3^{b}
Gill raker number	15.9^{a}	$16.7^{a,b}$	$16.8^{a,b}$	$16.1^{a,b}$	19.5^{d}	18.8^{d}	$16.8^{a,b}$	19.2^{d}	$17.3^{b,c}$	$18.2^{c,d}$
Lateral plate number	$6.1^{a,b}$	6.7^{b}	$6.6^{a,b}$	6.7^{b}	$6.2^{a,b}$	$6.1^{a,b}$	6.0^{a}	$6.3^{a,b}$	$6.4^{a,b}$	6.0^{a}
Body depth	14.5^{f}	13.6^{d}	$14.2^{e,f}$	$13.8^{d,e}$	12.4^{c}	11.6^{b}	12.7^{c}	12.5^{c}	11.0^{a}	10.6^{a}
Pelvic spine length	8.1^{a}	$8.7^{a,b}$	8.2^{a}	$9.0^{b,c}$	$9.3^{b,c}$	$9.2^{b,c}$	9.4^{c}	$9.3^{b,c}$	9.5^{c}	9.4^{c}
Upper jaw length	4.1^{c}	$3.8^{a,b,c}$	$4.0^{b,c}$	4.1^{c}	$3.7^{a,b}$	$3.7^{a,b}$	$3.9^{a,b,c}$	$3.7^{a,b}$	3.6^{a}	$3.7^{a,b}$
Pelvic girdle width	4.6^{e}	4.1^{d}	$4.5^{d,e}$	$4.3^{d,e}$	$3.1^{a,b}$	2.9^{a}	$3.2^{a,b,c}$	$3.5^{b,c}$	$3.3^{b,c}$	3.5^{c}

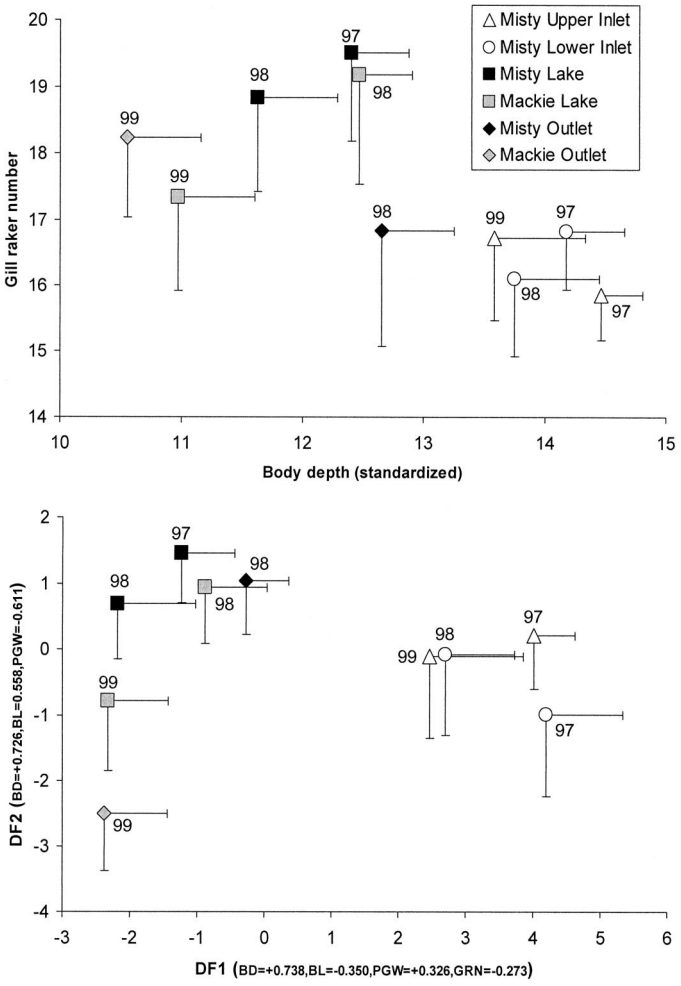

FIG. 2. Morphological differentiation among the different collections. The upper panel shows mean values for the number of gill rakers and standardized body depth in each collection (bars show standard deviations). The lower panel shows values at the group centroids for the first two discriminant functions (bars show standard deviations). Traits that load most heavily on each discriminant function are indicated in the axis labels. BD, standardized body depth; BL, body length; PGW, standardized pelvic girdle width; and GRN, number of gill rakers.

plying $b = 1.167$, standardized upper jaw length varied among collections ($F = 5.92$, $P < 0.001$) but without obvious trends among sites. Pelvic girdle width was positively correlated with body length ($F = 865.07$, $P < 0.001$) and slopes were heterogeneous ($F = 2.24$, $P = 0.020$). Applying $b = 1.955$, standardized pelvic girdle width varied among collections ($F = 61.81$, $P < 0.001$), with Misty Inlet fish having wider pelvic girdles than Misty Lake or Misty Outlet fish (Table 1).

Discriminant functions analysis revealed varying degrees of morphological separation among stickleback from the different sites. The first canonical function explained 71.1% of the variation and the second an additional 18.2%. The first function had standardized loadings of body length = −0.350, gill raker number = −0.273, lateral plate number = 0.114, body depth = 0.738, pelvic spine length = −0.203, upper jaw length = −0.082, and pelvic girdle width = 0.326. The second function had loadings of body length = 0.558, gill raker number = 0.130, lateral plate number = 0.027, body depth = 0.726, pelvic spine length = 0.048, upper jaw length = −0.082, and pelvic girdle width = −0.611. Five additional functions were extracted, each explaining 0.1–5.7% of the total variation. Using all seven functions, 164 of 261 fish (62.8%) were correctly classified back to their specific collection (site and year; Table 2). Of 87 fish collected from Misty Inlet, 49 (56.3%) were correctly classified back to their collection site (upper or lower). Of the 38 misclassifications, 36 (94.7%) were to the opposing inlet site (upper vs. lower). Of 115 fish collected from the two lakes, 78 (67.8%) were correctly classified back to their collection site (Misty or Mackie Lake). Of the 37 misclassifications, 24 (64.9%) were to the opposing lake (Misty vs. Mackie) and the other 13 were to outlet streams. Of the 59 fish collected from the two outlet streams, 50 (84.7%) were correctly classified back to their collection site. All nine misclassifications were to lakes (all but one to Mackie). Of the 261 total fish, 36 (13.8%) were classified to the wrong system (Misty vs. Mackie).

Total morphological divergence among sites within the Misty system (based on Euclidean distances, Table 3) was least between Misty Lower Inlet and Misty Upper Inlet (average $D_{cd} = 1.95$) and between Misty Lake and Misty Outlet ($D_{cd} = 2.33$). Divergence was greatest between Misty Lake and Misty Upper Inlet ($D_{cd} = 5.22$) and between Misty Lake and Misty Lower Inlet ($D_{cd} = 5.58$). Stickleback were fairly similar in the two lakes ($D_{cd} = 1.95$) and divergence between

TABLE 2. Results of discriminant functions analysis assigning individuals to different collections based on morphology (mean trait values listed in Table 1). The diagonal in the assigned collection columns indicates fish correctly classified back to their source collection. The collection to which fish from a particular source were most commonly misclassified is indicated in bold. The last three columns give the percentage of fish from a given collection correctly classified back to their specific collection (site and year specific), to their collection site (ignoring years), and to their general habitat type (inlet, lake, or outlet). Abbreviations for collections are described in the caption for Table 1.

Source collection	Assigned collection										Assignment success (%)		
	MUI97	MUI99	MLI97	MLI98	ML97	ML98	MO98	MaL98	MaL99	MaO99	Collection	Site	Habitat
MUI97	5	**1**		**1**							71	86	100
MUI99	1	8	8	**10**		1	1				28	31	93
MLI97	**4**	**4**	13								62	62	100
MLI98	4	**5**	1	20							67	70	100
ML97					17	4	1	**6**	1		59	72	97
ML98					**4**	19	2	2	3		63	77	93
MO98					1		22	**4**	2		76	76	76
MaL98					**6**	1	**6**	15		1	52	52	76
MaL99					1	**4**		2	17	3	63	70	89
MaO99									**2**	28	93	93	93

lake and outlet fish was slightly higher in the Mackie system (D_{cd} = 3.01) than in the Misty system (D_{cd} = 2.33).

Common-Garden Experiment

Although quantitative data on survival were not collected, survival was high, except for the lake × lake cross (five fish survived) and the lake × outlet cross (15 fish survived). Most of the mortality took place before hatching and was caused by fungus that infected some egg masses (a common occurrence in the laboratory). High mortality in only two specific crosses was probably not a consequence of the source populations because other crosses with lake and outlet fish had high survival. We also do not expect that selection in the laboratory had any effect on morphological variation because most mortality occurred before hatching. The following results demonstrate that several of the differences among wild populations had a genetic basis (differences among pure crosses were similar to those in nature, Table 4). Moreover, several of the differences appeared to have a strong contribution from additive genetic variation (hybrid crosses were intermediate between pure crosses, Table 4), whereas others appeared to have at least some contribution from nonadditive genetic variation (hybrid crosses were not intermediate, Table 4).

Body length varied among crosses (F = 14.77, $P < 0.001$) but is not considered further because they experienced different densities in the laboratory. However, we expect that variation in density did not effect metric traits or morphology after standardization to a common body size (this could not be tested directly because the same cross types were not reared at multiple densities). The number of gill rakers varied among crosses (F = 3.52, P = 0.005), showed trends among pure crosses similar to those in the wild (inlet < outlet < lake), and showed intermediacy of hybrid crosses (except lake × outlet). The number of lateral plates also varied among crosses (F = 4.54, P = 0.001), showed trends among pure crosses similar to those in the wild (inlet > outlet = lake), and showed intermediacy of hybrid crosses. Body depth was positively correlated with body length (F = 155.51, $P < 0.001$) and slopes were homogeneous among crosses (F = 0.32, P = 0.900). Applying the allometric coefficient of b = 0.946, standardized body depth varied among crosses (F = 48.16, $P < 0.001$), showed trends among pure crosses similar to those in the wild (lake < outlet < inlet), and showed intermediacy of hybrid crosses (except lake × outlet). Pelvic spine length was positively correlated with body length (F = 76.80, $P < 0.001$) and slopes were homogeneous (F = 1.03, P = 0.404). Applying b = 1.142, standardized spine

TABLE 3. Morphological distances (Euclidean) between each pair of collection sites. Values above the diagonal (D_z) are based on Z-scores for the original traits (four were standardized to a common body size). Values below the diagonal (D_{cd}) are based on discriminant function scores (see text). Estimates are presented as averages, with ranges of any pairwise (i.e., year-specific) estimates in parentheses. Abbreviations for collection sites are described in the caption for Table 1.

	MUI	MLI	ML	MO	MaL	MaO
MUI		2.36 (1.79–2.80)	4.69 (3.96–5.35)	3.81 (3.62–4.00)	4.35 (3.55–5.36)	4.65 (4.11–5.20)
MLI	1.95 (1.26–2.33)		5.13 (4.69–5.52)	4.24 (3.72–4.75)	4.74 (4.16–5.39)	4.96 (4.82–5.10)
ML	5.22 (4.10–6.38)	5.58 (4.52–6.64)		2.45 (2.30–2.60)	1.47 (0.69–2.11)	2.29 (2.04–2.53)
MO	4.05 (3.35–4.74)	4.40 (3.51–5.30)	2.33 (2.23–2.42)		2.45 (2.39–2.50)	2.54
MaL	5.08 (3.69–6.55)	5.39 (3.98–6.84)	1.95 (0.97–2.97)	2.69 (2.32–3.07)		2.32 (2.13–2.52)
MaO	6.26 (5.45–7.06)	6.28 (5.76–6.80)	3.71 (3.27–4.16)	4.38	3.01 (2.17–3.86)	

TABLE 4. Average morphological measurements for different cross types from the Misty system (inlet fish were from Misty Lower Inlet) raised in a common laboratory environment. Body depth, pelvic spine length, upper jaw length, and pelvic girdle width are standardized to a common body length of 42.1 mm. Homogenous subsets of crosses based on Tukey tests are indicated with letter superscripts.

	Inlet × inlet	Inlet × lake	Lake × lake	Lake × outlet	Outlet × outlet	Outlet × inlet
N	28	29	5	15	30	30
Body length	40.8[a,b]	41.6[a,b]	43.8[b]	47.7[c]	43.0[a,b]	40.0[a]
Gill raker number	18.0[a,b]	19.0[b]	19.2[b]	17.8[a]	18.6[a,b]	18.3[a,b]
Left plate number	6.5[b]	6.0[a,b]	5.8[a]	6.0[a,b]	6.1[a,b]	6.4[b]
Body depth	10.5[d]	9.6[b,c]	8.4[a]	9.6[b,c]	9.3[b]	9.7[c]
Pelvic spine length	7.9[b]	8.4[c,d]	6.9[a]	8.4[c,d]	8.3[b,c]	8.8[d]
Upper jaw length	2.8[b]	2.6[a,b]	2.7[a,b]	2.7[a,b]	2.5[a]	2.6[a,b]
Pelvic girdle width	2.3[b]	2.1[a]	2.4[b]	2.4[b]	2.1[a]	2.4[b]

length differed among crosses ($F = 22.32$, $P < 0.001$) but did not show trends among pure crosses similar to those in the wild and did not show intermediacy of hybrid crosses. Upper jaw length was positively correlated with body length ($F = 22.03$, $P < 0.001$) and slopes were homogeneous ($F = 0.85$, $P = 0.517$). Applying $b = 1.036$, standardized upper jaw length varied among crosses ($F = 3.78$, $P = 0.003$) and showed intermediacy of hybrid crosses. Pelvic girdle width was positively correlated with body length ($F = 75.12$, $P < 0.001$) and slopes were homogeneous ($F = 0.71$, $P = 0.619$). Applying $b = 1.492$, standardized pelvic girdle width varied among collections ($F = 12.66$, $P < 0.001$) but did not show trends among pure crosses similar to those in the wild and did not show intermediacy of hybrid crosses.

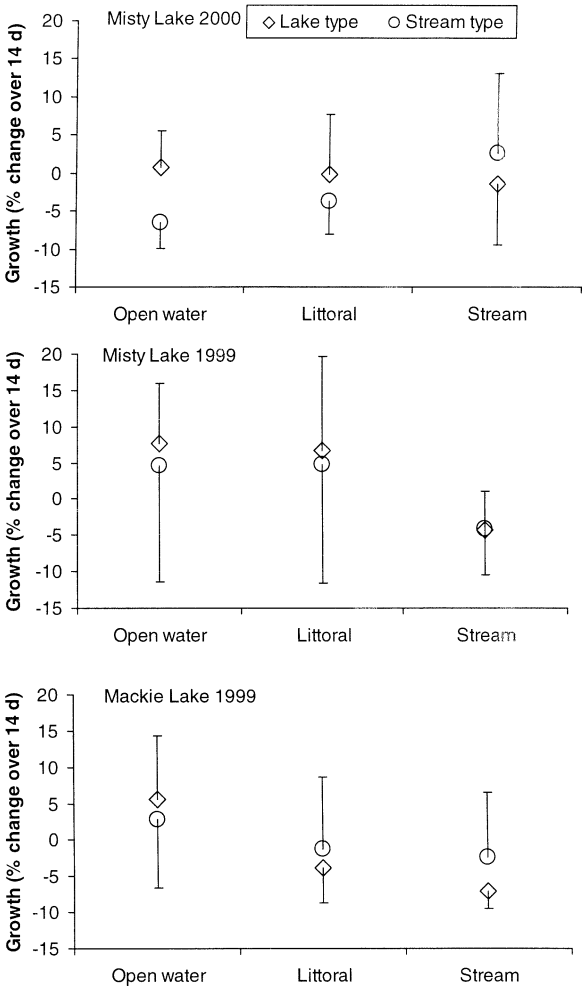

FIG. 3. Growth rate variation (means and standard deviations) in the three experiments comparing lake fish (lake type) and stream fish (stream type) in each of three different enclosure types (open-water, littoral, stream). Results are shown for Misty Lake and Misty Lower Inlet fish in 2000 (top panel) and 1999 (middle panel), and Mackie Lake and Mackie Outlet fish in 1999 (bottom panel).

Reciprocal Transplant Enclosure Experiments

For Misty Lake in 2000, we recovered 20 lake and 21 stream fish from the open-water enclosures (18 complete pairs), 18 lake and 17 stream fish from the littoral enclosures (13 pairs), and 15 lake and 18 stream fish from the stream enclosures (11 pairs). Lake fish grew better (gained more mass or lost less mass) than stream fish in the open-water enclosures (paired $t = 5.430$, $P < 0.001$) and, to a lesser degree, in the littoral enclosures (paired $t = 1.694$, $P = 0.058$; Fig. 3). In the stream enclosures, estimated relative growth was higher for stream fish than for lake fish (Fig. 3), but not significantly so (paired $t = 1.068$, $P = 0.155$). Home fish thus grew better than foreign fish within each environment, although statistical support varied. Also, each fish type grew better in its home environment than in foreign environments ($F = 6.111$, $P = 0.005$; Fig. 3). A post hoc Tukey test showed that this result was largely driven by differences between stream enclosures and both open-water ($P = 0.003$) and littoral ($P = 0.087$) enclosures, with no difference between open-water and littoral enclosures ($P = 0.450$).

For Misty Lake in 1999, we recovered 17 lake and 20 stream fish from the open-water enclosures (15 pairs), 13 lake and 16 stream fish from the littoral enclosures (seven pairs), and 11 lake and 17 stream fish from the stream enclosures (nine pairs). Estimated growth was marginally higher for home fish than for foreign fish in each enclosure type (Fig. 3) but never significantly so (open-water, paired $t = 0.970$, $P = 0.174$; littoral, paired $t = 0.301$, $P = 0.387$; stream, paired $t = 0.051$, $P = 0.480$). Estimated growth was higher in home than in foreign environments for lake but not stream fish, and so the overall comparison of home versus foreign environments was not significant ($F = 0.189$, $P = 0.829$).

For Mackie Lake in 1999, we recovered 20 lake and 23 stream fish from the open-water enclosures (19 pairs), 19 lake and 19 stream fish from the littoral enclosures (17 pairs), and 14 lake and 17 stream fish from the stream enclosures

TABLE 5. Counts of stickleback released and recaptured in a section of the Misty Inlet stream. Lake and stream fish were released into a semi-isolated section and then recaptured using minnow traps and seine nets approximately two weeks later.

	Stream fish	Lake fish
1999 experiment		
Released	136	136
Recaptured at release site	44	6
Recaptured below release site	13	29
Total recaptures	57 (41.9%)	35 (25.7%)
Recaptured above release site in 2000	2	0
Recaptured at release site in 2000	7	0
Recaptured below release site in 2000	2	0
2000 experiment		
Released	135	134
Recaptured above release site	9	1
Recaptured at release site	43	21
Recaptured below release site	15	25
Total recaptures	67 (49.6%)	47 (35.1%)

(eight pairs). Estimated growth was higher for home fish than for foreign fish in the open-water and stream enclosures (Fig. 3) but not in the littoral enclosures, and none of the differences were significant (open-water, paired $t = 0.984$, $P = 0.169$; littoral, paired $t = -0.892$, $P = 0.193$; stream, paired $t = 1.059$, $P = 0.162$). Estimated growth was higher in home than in foreign environments for lake but not stream fish, and so the overall comparison of home versus foreign environments was not significant ($F = 1.325$, $P = 0.277$).

Release-Recapture Experiments

These experiments could not separate differential survival from differential emigration but they nevertheless suggested that stream fish are better suited for life in the stream than are lake fish (Table 5). First, we recaptured more stream than lake fish in 1999 (Fisher's exact test, two-tailed $P = 0.007$) and 2000 (Fisher's, $P = 0.019$). Second, downstream movement was more common in lake fish than stream fish in 1999 (Fisher's, $P < 0.001$) and 2000 (Fisher's, $P < 0.001$). Third, all fish captured in 2000 that had been released in 1999 were stream fish ($N = 11$). Fourth, only 16% (five of 31) of the unclipped fish captured in 2000 (progeny of fish released in 1999) had mtDNA of the ENA clade. This suggests that few of them (if any) had lake mothers because the ENA clade has a frequency of 95.6% in Misty Lake and 32.5% in Misty Lower Inlet (the sources of fish for the experiment). These results suggest that lake fish were more likely to die or emigrate, and that lake females were less likely to produce offspring.

Population Genetics

Frequencies of the TNP clade and the ENA clade did not differ significantly among years within sites ($0.8 > P > 0.06$; Table 6) but did differ significantly for all pairwise comparisons among sites ($P < 0.001$ for each; Table 6). In general, the TNP clade was nearly fixed in Misty Upper Inlet (95.5%), dominant in Misty Lower Inlet (67.5%), less common in Misty Outlet (20.5%), and rare in Misty Lake (4.4%). All Mackie samples were fixed for the ENA clade.

TABLE 6. Frequencies of the two major clades of mitochondrial DNA (Trans-North Pacific, TNP, and Euro-North American, ENA) in the various collections as assayed with restriction fragment polymorphisms (using *Nsi* I) of the cytochrome *b* gene. No significant frequency differences were present among years within sites, but frequencies differed among all sites within the Misty system.

Sites	Year	TNP clade	ENA clade	Percent TNP
Misty Lake	1993	1	24	4.0
	1995	1	15	6.3
	1997	1	19	5.0
	1998	1	28	3.4
	Total	4	86	4.4
Misty Lower Inlet	1993	4	2	66.7
	1994	39	11	78.0
	1996	3	5	37.5
	1997	11	9	55.0
	1998	20	10	66.7
	Total	77	37	67.5
Misty Upper Inlet	1996	4	0	100.0
	1997	10	0	100.0
	1999	28	2	93.3
	Total	42	2	95.5
Misty Outlet	1993	4	11	26.7
	1994	6	24	20.0
	1998	5	23	17.9
	Total	15	58	20.5
Mackie Lake	1999	0	30	0.0
Mackie Outlet	1999	0	30	0.0

Only three of the 60 tests for pairwise linkage disequilibrium between microsatellite loci were significant ($P < 0.05$), and none remained significant after sequential Bonferroni corrections (Table 7). Hardy-Weinberg equilibrium was rejected in five of 36 possible tests but only one remained significant after sequential Bonferroni corrections (*Gacu*9 in Misty Upper Inlet, Table 7). Microsatellite variation was usually substantial within collections (Table 7), but fish from Mackie Lake and Mackie Outlet were fixed for the same alleles at two of five loci. Accordingly, Mackie system fish were highly distinct from Misty system fish ($P < 0.001$; $F_{ST} = 0.560$–0.696; Fig. 4). Allelic frequency differences at the three variable loci within the Mackie system were nevertheless sufficient to discriminate between the lake and outlet fish ($P < 0.001$; $F_{ST} = 0.102$).

Within the Misty system, all pairs of collections (site- and year-specific) differed significantly in allelic frequencies at microsatellite loci ($P < 0.010$). Similarly, pairwise F_{ST}-values (Table 8) were all significantly greater than zero ($P < 0.001$). Differentiation between years within sites was small ($F_{ST} = 0.023$–0.030), whereas differentiation among sites ranged from small (Misty Lake vs. Misty Outlet, $F_{ST} = 0.005$–0.046) to moderate (Misty Lower Inlet vs. Misty Lake, $F_{ST} = 0.129$–0.157) to large (Misty Upper Inlet vs. Misty Lake, $F_{ST} = 0.289$–0.345). In general, the Misty Lake and Misty Outlet collections clustered together, and were quite distinct from the Misty Lower Inlet and Misty Upper Inlet collections (98% bootstrap support; Fig. 4). Misty Lower Inlet and Misty Upper Inlet were also quite distinct from each other ($F_{ST} = 0.120$–0.191; 99% bootstrap support).

Assignment tests based on multilocus genotypes revealed

TABLE 7. Microsatellite variation within collections of stickleback. Data include sample sizes (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosities, and allele size ranges in base pairs (ASR). Locus *Cir*51 is from Rico et al. (1993) and loci *Gacu*4, 7, 9, 14 are from Taylor (1998). Abbreviations for collections are described in the caption for Table 1.

Locus		MUI99	MLI97	MLI98	MO98	MO99	ML97	ML98	MaL99	MaO99
*Cir*51	N	40	20	30	29	26	27	30	30	29
	N_a	1	6	8	5	11	11	7	7	5
	ASR	191	177–201	177–205	177–191	177–201	177–231	177–197	193–203	195–205
	H_o/H_e	0/0	0.39/0.40	0.37/0.43	0.45/0.43	0.63/0.58	0.52/0.52	0.5/0.52	0.77/0.81	0.66/0.64
*Gacu*4	N	39	20	29	26	28	30	30	30	29
	N_a	4	3	7	8	8	9	10	1	1
	ASR	115–141	115–141	115–147	115–139	115–135	115–141	115–141	127	127
	H_o/H_e	0.75/0.79	0.80/0.80	0.72/0.82	0.46/0.46	0.64/0.71	0.54/0.59	0.67/0.66	0/0	0/0
*Gacu*7	N	39	20	29	29	29	25	30	30	29
	N_a	3	5	7	10	11	11	7	2	1
	ASR	122–154	122–154	118–158	122–158	122–160	122–156	122–158	122–124	122
	H_o/H_e	0.10/0.09	0.60/0.63	0.52/0.62	0.79/0.83	0.79/0.82	0.80/0.84	0.63/0.77	0.03/0.03	0/0
*Gacu*9	N	40	19	28	26	30	30	30	30	30
	N_a	4	2	3	6	5	6	5	3	3
	ASR	168–174	168–174	168–174	164–174	164–172	168–180	168–174	178–184	178–182
	H_o/H_e	0.10/0.26*	0.26/0.46	0.47/0.58	0.54/0.65	0.76/0.67	0.61/0.71	0.40/0.60	0.13/0.17	0.10/0.09
*Gacu*14	N	39	20	30	26	30	29	30	30	29
	N_a	3	1	1	3	5	4	4	1	2
	ASR	107–111	111	111	107–111	107–125	107–111	107–115	115	115–117
	H_o/H_e	0.24/0.19	0/0	0/0	0.23/0.21	0.34/0.47	0.38/0.33	0.20/0.18	0/0	0.04/0.04

* The only significant deviation from Hardy-Weinburg equilibrium after corrections for multiple tests.

similar patterns of differentiation within the Misty system (Table 9). In total, 113 of 210 fish (53.8%) were correctly classified back to their specific collection (site and year). Of 90 fish collected from Misty Inlet, 69 (76.7%) were correctly classified back to their collection site (upper or lower). Of the 21 misclassifications, 11 (52.4%) were to the opposing inlet site (upper vs. lower). Of 60 fish collected from Misty Lake, 38 (63.3%) were correctly classified back to Misty Lake. Of the 22 misclassifications, 19 (86.4%) were to Misty Outlet. Of 60 fish collected from Misty Outlet, 38 (63.3%) were correctly classified back to Misty Outlet. Of the 22 misclassifications, 17 (77.3%) were to Misty Lake. Within the Mackie system in 1999, 21 of 30 lake fish and 23 of 30 outlet fish were correctly classified back to their collection site. If all collections were included into one big assignment test, all fish were correctly classified back to their overall system (Misty or Mackie).

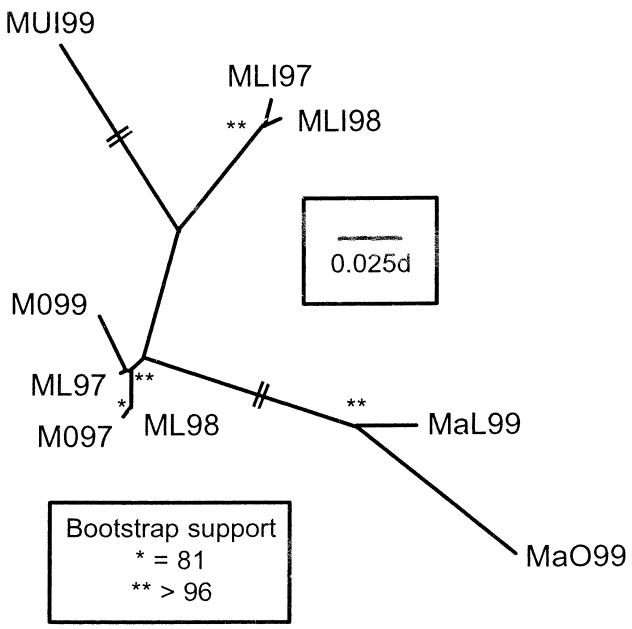

FIG. 4. Unrooted neighbor-joining tree of genetic similarity among the collections. The genetic distance used was Reynolds et al.'s (1983) coancestry coefficient and is based on allelic frequency variation at five microsatellite loci. Numbers at branch points represent bootstrap resampling confidence percentages (N = 1000 in resampling analyses). Branch lengths to MUI99 and the node joining the Mackie Lake samples (MaL99, MaO99) have been reduced to 0.05 and 0.10, respectively, of their actual values to improve tree visualization.

Gene Flow and Its Effects

The estimated effective number of migrants (N_em) between Misty Lake and each of the three stream sites depended on the estimation method and the particular pair. However, the different pairings showed the same pattern of variation regardless of the method (except for a slight deviation for $N_em_{Slatkin}$): lowest between Misty Lake and Misty Upper Inlet (N_em_{Wright} = 0.54, $N_em_{Takahata}$ = 0.14, $N_em_{Slatkin}$ = 1.88, N_em_{Beerli} = 2.62), slightly higher between Misty Lake and Misty Lower Inlet (N_em_{Wright} = 1.59, $N_em_{Takahata}$ = 0.40, $N_em_{Slatkin}$ = 1.59, N_em_{Beerli} = 3.43), and much higher between Misty Lake and Misty Outlet (N_em_{Wright} = 18.23, $N_em_{Takahata}$ = 4.56, $N_em_{Slatkin}$ = 2.97, N_em_{Beerli} = 15.13). Much of the variation between estimation methods is readily explainable. $N_em_{Takahata}$ estimates are lower than N_em_{Wright} estimates because the former accounts for finite numbers of populations: the amount of gene flow necessary to sustain a given amount of divergence decreases as the number of populations decreases (Takahata 1983). $N_em_{Slatkin}$ estimates vary the least among population pairs because this a property of Slatkin's method relative to those based on θ as an estimator of F_{ST} (Slatkin and Barton 1989). N_em_{Beerli} estimates tend to be high-

TABLE 8. Pairwise measures of genetic differentiation among all collections genotyped at five microsatellite loci. Reynolds et al.'s (1983) genetic distances are above the diagonal and $F_{ST}(\theta)$ values are below. All F_{ST} values are significantly greater than zero based on permutation analyses ($P < 0.010$ for each). Abbreviations for collections are described in the caption for Table 1.

	MUI99	MLI97	MLI98	MO98	MO99	ML97	ML98	MaL99	MaO99
MUI99		0.213	0.131	0.404	0.421	0.342	0.426	1.121	1.192
MLI97	0.191		0.026	0.155	0.170	0.139	0.173	0.985	1.056
MLI98	0.120	0.023		0.141	0.159	0.133	0.151	0.930	0.989
MO98	0.330	0.142	0.130		0.025	0.026	0.007	1.030	1.090
MO99	0.342	0.156	0.145	0.023		0.048	0.033	0.823	0.871
ML97	0.289	0.129	0.123	0.024	0.046		0.032	0.893	0.945
ML98	0.345	0.157	0.138	0.005	0.031	0.029		0.949	1.001
MaL99	0.674	0.626	0.605	0.643	0.560	0.590	0.613		0.108
MaO99	0.696	0.652	0.627	0.664	0.581	0.611	0.632	0.102	

est because they represent the total number of migrants exchanged between populations, whereas the others represent the average number of migrants (Beerli and Felsenstein 1999). As noted above, the $N_e m_{Beerli}$ estimates are probably most accurate and the others are provided simply to allow comparison with other studies.

The absolute amount of gene flow (m) among population pairs followed the same pattern as the $N_e m$ estimates: lowest for Misty Lake versus Misty Upper Inlet ($m_{Beerli} = 0.00040$), slightly higher for Misty Lake versus Misty Lower Inlet ($m_{Beerli} = 0.00047$), and much higher for Misty Lake versus Misty Outlet ($m_{Beerli} = 0.00267$). (Note: $N_{eBeerli}$ estimates were Misty Lake = 8288, Misty Upper Inlet = 5018, Misty Lower Inlet = 5561, and Misty Outlet = 5134.) For comparison, we also estimated m using the other $N_e m$ estimates together with heterozygosity-based estimates of N_e (Misty Lake = 13,451, Misty Upper Inlet = 3624, Misty Lower Inlet = 9029, and Misty Outlet = 14,616). The resulting m estimates were lower than the m_{Beerli} estimates but followed the same general pattern (again except for a slight deviation for $m_{Slatkin}$): lowest for Misty Lake versus Misty Upper Inlet ($m_{Wright} = 0.00006$, $m_{Takahata} = 0.00002$, $m_{Slatkin} = 0.00022$), slightly higher for Misty Lake versus Misty Lower Inlet ($m_{Wright} = 0.00014$, $m_{Takahata} = 0.00004$, $m_{Slatkin} = 0.00014$), and much higher for Misty Lake versus Misty Outlet ($m_{Wright} = 0.00130$, $m_{Takahata} = 0.00032$, $m_{Slatkin} = 0.00021$). The absolute level of gene flow between Misty Lake and Misty Outlet (m_{Beerli}) was estimated to constrain adaptive divergence to 96% of its optimum if stabilizing selection is strong, 75% of its optimum if stabilizing selection is moderate, and 53% of its optimum if stabilizing selection is weak. For comparison, m estimates from the other methods would suggest that the constraining role of gene flow was slightly weaker (m_{Wright} = 98%, 86%, and 69%) or considerably weaker (m_{Takaha} = 99%, 96%, and 90%; $m_{Slatkin}$ = 100%, 97%, and 93%).

DISCUSSION

Morphology and Adaptation: Misty Lake versus Misty Inlet

Misty Lake fish had shallower bodies and more gill rakers than Misty Inlet fish (Table 1, Fig. 2), differences that match those expected to improve feeding performance in home environments (see introduction). Our common-garden experiments (and those of Lavin and McPhail 1993) confirmed that these phenotypic differences have a genetic basis (Table 4), driven at least in part by additive genetic variation (because hybrids were intermediate to pure types). Similar relationships between these traits and foraging environments have previously been documented for Misty Lake versus Misty Inlet (Lavin and McPhail 1993), other parapatric lake-stream pairs (Moodie 1972a; Reimchen et al. 1985), allopatric lake versus stream stickleback (Hagen and Gilbertson 1972; Gross and Anderson 1984), sympatric benthic versus limnetic stickleback (Schluter and McPhail 1992), and numerous other sympatric pairs of fish species (Schluter 1996). There seems little doubt that these differences are adaptive.

Misty Lake and Misty Inlet fish also differed (not always significantly) in three armor traits: Inlet fish had more lateral plates, shorter pelvic spines, and wider pelvic girdles (Table

TABLE 9. Results of assignment tests classifying individuals to different collections in the Misty Lake system based on multilocus genotypes. The diagonal in the assigned collection columns indicates fish correctly classified back to their source collection. The collection to which fish from a particular source were most commonly misclassified is indicated in bold. The last three columns give the percentage of fish from a given collection correctly classified back to their specific collection (site and year specific), their collection site (ignoring years), and their general habitat type (inlet, lake, or outlet). Abbreviations for collections are described in the caption for Table 1.

Source collection	Assigned collection							Assignment success (%)		
	MUI99	MLI97	MLI98	MO98	MO99	ML97	ML98	Collection	Site	Habitat
MUI99	33	**4**					2	85	85	95
MLI97	**3**	11	2	2		2		55	65	80
MLI98	3	**8**	15	4				50	77	87
MO98	1	1	3	7	5	2	**11**	23	40	40
MO99				**8**	18	4		60	87	87
ML97	1	1		**6**	3	15	4	50	63	63
ML98			1	**8**	2	5	14	47	63	63

1). The differences in plate number and spine length parallel those documented by Lavin and McPhail (1993) in the Misty system (they did not measure pelvic girdles). Lake and stream fish from the Mayer and Drizzle systems differ in the same direction for spine length (shorter in the inlet stream) but in the opposite direction for plate number (more in the lake; Moodie 1972a,b; Reimchen et al. 1985). Lavin and McPhail (1993) found that the differences in spine length and lateral plates were maintained under common rearing, but this was true in our study only for lateral plates (Table 4). Moreover, spine length and girdle width in hybrid crosses were not intermediate to pure crosses (Table 4). Divergence in these traits may thus involve a complex interaction between environmental effects and genetic variation (additive and nonadditive). Armor traits diverge among populations in response to selection by predators (Hagen and Gilbertson 1972; Moodie 1972b; Reimchen 1994) but the distribution of predators in the Misty system is not known. Owing to this ambiguity about selection and genetic variation, we do not further interpret differences in armor traits.

If the observed morphological differences reflect adaptive divergence, Misty Lake fish should perform best in lake environments (open-water and littoral) and Misty Inlet fish should perform best in stream environments. We tested this prediction using reciprocal transplant enclosure experiments (to measure growth) and release-recaptures experiments in the stream (to measure survival and emigration). Variation in growth was generally consistent with the adaptive prediction. Misty Lake fish tended to grow at their highest rates in open-water enclosures and at their lowest rates in stream enclosures, whereas the converse was true for Misty Inlet fish in one year (Fig. 3). Within lake enclosures (open-water and littoral), Misty Lake fish grew at higher rates than Misty Inlet fish (significant in one year), whereas the converse was true in stream enclosures (not significant in either year, Fig. 3). If these patterns reflect adaptive divergence, they should weaken when lake and stream fish are less divergent. This proved to be the case in Mackie Lake, where growth rates did not differ significantly between lake and stream fish in any of the enclosure types, and stream fish actually tended to grow better than lake fish in the littoral enclosures (Fig. 3). The release-recapture experiments were also consistent with adaptive divergence in the Misty system, although we cannot separate survival from emigration. First, lake fish were more likely than stream fish to move downstream and were less likely to be recaptured (Table 5). Second, the only fish released in 1999 that were recaptured in 2000 were stream fish. Third, the offspring of experimental fish did not appear to have lake mothers (based on mtDNA analysis). Thus, even though the results of individual experiments were not necessarily conclusive, the combined evidence suggests that natural selection has caused the adaptive divergence of lake and stream stickleback within the Misty system.

The performance differences between fish from Misty Lake and Misty Lower Inlet (each performed best in its home environment) are probably the result of genetic differences in traits such as body depth and gill rakers. A possible alternative is prior experience. Our experimental fish were captured from the wild and held in coolers for less than a day before being introduced into the experimental enclosures or release site. It is therefore possible that previous experience increased the success of fish tested in their home environments, relative to those tested in foreign environments. However, analogous experiments conducted on benthic and limnetic stickleback have shown that prior experience does not drive performance differences. Those experiments used the same enclosures that we used (open-water and littoral) and first tested wild-caught fish (Schluter 1995) and then fish reared from eggs in the laboratory (Hatfield and Schluter 1999). The two experiments yielded similar results (better growth by each type in its home environment), suggesting that performance differences are largely innate.

Although Misty Lake and Misty Lower Inlet fish performed best in their home environments and differed in morphological traits that contribute to performance, growth was poor overall and the differences were subtle. Many of the fish in our enclosures lost mass during the experiment (Fig. 3), in contrast to experiments conducted using the same enclosures (open-water and littoral) for the same length of time with benthic and limnetic stickleback (Schluter 1995; Hatfield and Schluter 1999; Rundle 2002). One reason for reduced growth in our experiments may be our use of two fish per enclosure rather than the single fish used in the benthic-limnetic experiments. We also used some larger, older fish, for which growth rates would be lower. Growth was particularly poor in our stream enclosures, perhaps because they did not fully replicate the environment of stream fish (such experiments have not previously been conducted in streams).

Gene Flow and Adaptive Divergence

If gene flow constrains the ability of populations to diverge in response to natural selection, then less morphologically divergent populations should also exchange more genes (Endler 1977; García-Ramos and Kirkpatrick 1997; Hendry et al. 2001). Here we examine the extent of morphological and genetic divergence between lake stickleback and different stream stickleback populations within the Misty system. We then evaluate relative and absolute levels of gene flow for the various lake-stream contrasts, asking whether adaptation in any population appears constrained by gene flow. For this analysis, we assume that any gene flow that might be taking place is from the lake into the streams rather than the inverse (for simplicity, and because the lake population is much larger). This assumption is particularly appropriate for the outlet because it should be easier for lake fish to enter the outlet than to enter the inlet (because of the direction of water flow).

Morphological divergence between lake and stream stickleback was greatest for Misty Lake versus Misty Inlet (upper and lower). This pattern was evident for the two morphological traits related to foraging (body depth and gill rakers) and for all traits combined (Tables 1, 3; Fig. 2). Discriminant functions analysis misclassified very few fish between these sites (one of 146; Table 2). Morphological divergence was least for Misty Lake versus Misty Outlet (Tables 1, 3; Fig. 2) but they nevertheless remained distinct on the basis of discriminant functions (four of 88 fish misclassified between these sites; Table 2). Misty Outlet fish were intermediate between Misty Lake and Misty Inlet fish (although closer to Misty Lake) with respect to body depth, gill raker number,

and all traits combined (Fig. 2). Remarkably similar results in all these respects were obtained in Reimchen et al.'s (1985) morphological analysis of Drizzle Lake, Drizzle Inlet, and Drizzle Outlet stickleback, suggesting a general trend for inlet populations to be more divergent than outlet populations. Common-garden experiments confirmed that at least some of the morphological differences within the Misty system have a genetic basis (Table 4). However, the precise nature of the genetic variation is not clear because hybrids between Misty Lake and Misty Outlet were often not intermediate to pure crosses (Table 4). Mackie Lake and Mackie Outlet showed roughly similar divergence to that between Misty Lake and Misty Outlet (Table 1, Fig. 2).

Genetic divergence between lake and stream fish within the Misty system was greatest for Misty Lake versus Misty Upper Inlet. This was evident in nearly opposite mtDNA clade frequencies (Table 6), high levels of genetic differentiation at microsatellite loci (Table 8), and very low misclassification in assignment tests (4.3% between these sites; Table 9). These results closely match those from another stickleback system, where inlet and lake populations showed similar levels of genetic divergence at microsatellites (Schleswig-Holstein, Germany, overall lake vs. stream $F_{ST} = 0.18$; Reusch et al. 2001). Genetic divergence was slightly lower, but still high, between Misty Lake and Misty Lower Inlet, and was relatively low between Misty Lake and Misty Outlet (Tables 6, 8, 9). In fact, Misty Lake and Misty Outlet fish were less genetically distinct than even Mackie Lake and Mackie Outlet fish. Levels of gene flow estimated from these genetic data were highest between Misty Lake and Misty Outlet, lowest between Misty Lake and Misty Upper Inlet, and intermediate between Misty Lake and Misty Lower Inlet.

Gene flow in the Misty system appears to constrain adaptive divergence for one stream population (outlet) but not another (inlet). Evidence that adaptive divergence of the outlet population is constrained by gene flow comes first from the observation that gene flow was about 5.0 times higher and total morphological divergence about 2.5 times lower between outlet and lake fish than between inlet and lake fish. These relative differences are consistent with the prediction that populations exchanging more genes will be less morphologically distinct. We estimated the absolute rate of gene flow between Misty Lake and Misty Outlet to be roughly $m = 0.0027$. We then used this value for $\hat{m}$ in the theoretical equations of Hendry et al. (2001) to evaluate how adaptive divergence might be constrained in the outlet. This heuristic analysis suggested that adaptive divergence would be at 96% of the optimum if stabilizing selection is strong, 75% if stabilizing selection is moderate, and 53% if stabilizing selection is weak. These results suggest that observed gene flow from the lake into the outlet could theoretically explain the reduced morphological divergence of outlet fish. This last analysis is not definitive because it makes a number of assumptions and requires the (imprecise) estimation of several parameters.

Evidence that adaptive divergence of the inlet population is not constrained by gene flow comes from the observation that although gene flow was lower from the lake into the upper inlet than into the lower inlet, the absolute amount of morphological divergence was similar for the two sites (Table 3, Fig. 2). This suggests that inlet fish at different sites can reach a similar morphological equilibrium in response to natural selection, despite variation in the amount of gene flow from the lake. The most likely explanation for this observation is that gene flow is extremely low from the lake into even the lower inlet ($m = 0.00048$). Perhaps stabilizing selection is also stronger in the inlet than in the outlet, which would reduce the effects of gene flow on adaptive divergence in the inlet. Samples from nearer to the lake may be necessary before finding a group of inlet stickleback where gene flow constrains adaptation.

Alternative 1: Natural Selection and Reproductive Isolation

We have interpreted variation in the extent of adaptive divergence between lake and stream stickleback as a consequence of gene flow: Gene flow into the outlet compromises divergence. An alternative explanation is that the strength of divergent selection is less between the outlet and the lake than between the inlet and the lake. If so, the reproductive success of lake fish entering the inlet may be less than that of lake fish entering the outlet, owing to selection against immigrants (and hybrids) and perhaps to assortative mating. Such variation in ecologically dependent reproductive isolation (Schluter 1996; Hendry et al. 2000; Rundle and Whitlock 2001; Rundle 2002) could theoretically lead to lower gene flow into the inlet than into the outlet, even if both populations receive similar proportions of immigrants from the lake. In this scenario, cause and effect are reversed, and it is the amount of divergent adaptation that constrains gene flow, rather than the other way around. In truth, both processes can operate in a positive feedback loop: Low gene flow may allow adaptive divergence, which further reduces gene flow by increasing reproductive isolation.

Ecologically dependent reproductive isolation could potentially be higher in the inlet than in the outlet because (1) the outlet is downstream of the lake and might therefore have some lakelike features, (2) lake fish are more morphologically divergent from inlet fish than from outlet fish, and (3) experiments with lake and inlet stickleback in Mayer Lake (Moodie 1972a) and Drizzle Lake (Stinson 1983) suggest that mate preference could reduce heterotypic mating. Moreover, in another postglacial stickleback system (Schleswig-Holstein, Germany), inlet populations clustered together and separately from nearby lake populations in an analysis of microsatellite variation, suggesting that gene flow is higher among populations in similar environments (Reusch et al. 2001). However, it also seems likely that gene flow directly constrains adaptive divergence in Misty Outlet. First, although the inlet and outlet fish differ dramatically in adaptive traits, their environments are not that different (the outlet is still much more similar to the inlet than to the lake). Second, less gene flow into the inlet than into the outlet is consistent with the expectation that stickleback will have more trouble entering the inlet than the outlet, simply because it is more difficult to move upstream than downstream. Indeed, our release-recapture experiment showed that lake fish were much more likely to move downstream than upstream when placed in a stream environment (Table 5). Third, the absolute amount of gene flow into the outlet, relative to the inlet, is theoret-

ically consistent with the reduced morphological divergence of outlet fish. Future work should attempt to disentangle the relative influences of gene flow and ecologically dependent reproductive isolation within this system.

Alternative 2: Nonequilibrium Conditions and Historical Origins

Northern Vancouver Island was glaciated during the Pleistocene, and Misty Lake appears to have existed in its present location for about 12,000 years (Walker and Mathewes 1989). Different stickleback populations within the Misty system could have arisen in two ways: (1) the system could have been colonized by two separate lineages that segregated into different habitats; or (2) the system could have been colonized by a single lineage that subsequently diverged into stream and lake forms. The ENA clade of mtDNA, which predominates in Misty Lake (95.6%) and Misty Outlet (79.5%), shows approximately 2.5% sequence divergence from the TNP clade (Thompson et al. 1997), which predominates in Misty Inlet (95.5% in the upper inlet, 67.5% in the lower inlet; Table 6). This suggests that the ancestors of the Misty Inlet and Misty Lake populations evolved separately for more than 1 million years before coming into secondary contact within the Misty system. Alternatively, variation in clade frequencies could reflect incomplete lineage sorting after the Misty system was colonized by a single population polymorphic for the two mtDNA clades (Thompson et al. 1997). If the first scenario is true, we need to evaluate whether different origins might have contributed to the observed morphological or genetic differences or to reproductive isolation. If either scenario is true, we need to determine if the system has approached an equilibrium.

For traits subject to natural selection (morphology), equilibrium conditions have probably been reached, and divergence will reflect current selection rather than different ancestral origins. First, lake and stream fish in different systems (Misty, Mayer, Drizzle) have evolved similar sets of traits despite their often varying mtDNA clades (see discussion in Thompson et al. 1997). Second, either mtDNA clade can predominate in either lakes or streams in different locations (Deagle et al. 1996; Thompson et al. 1997; E. B. Taylor, unpubl. data). Third, an equilibrium between current selection and gene flow would be reached very quickly: Even assuming conservative estimates for the strength of stabilizing selection ($\omega^2 = 100$) and gene flow ($m = 0.004$), it should take only 682 generations to evolve 90% of the distance to equilibrium (using equation 13 in Hendry et al. [2001], $G = 0.3$, $P = 1.0$). Even under these restrictive conditions, an equilibrium would be approached within 1000 years (most stickleback mature after one year and very few live past two years; Baker 1994).

For traits not under selection (DNA microsatellites), we cannot be as certain of equilibrium conditions or the relative role of different origins. The scenario where the stream and lake sites were colonized by different lineages was evaluated using Whitlock's (1992) equation for the time required for genetic variance to decay half way to equilibrium: $t = \ln(1/2)/\ln[(1 - m)^2(1 - (2N_e)^{-1})]$, where m is the rate of gene flow (average into each population) and N_e is the average effective population size. This half-life rate of decay to equilibrium in the Misty system (using $m_{Beerli}/2$ and the average $N_{eBeerli}$) might therefore be on the order of 251 generations (lake vs. outlet), 1262 generations (lake vs. lower inlet), and 1498 generations (lake vs. upper inlet). If the above half-life estimates are correct, and if both lineages colonized the system soon after deglaciation, the observed genetic differences likely approximate current equilibrium population struture rather than different origins. If, however, the true N_e is higher, the true m is lower, or at least one of the lineages colonized the system well after deglaciation, an equilibrium may not have been reached and historical influences may still be important. If so, our estimates of N_em, m, and the constraint gene flow places on divergence are all too low (particularly for the inlet stream, which would take the longest to reach equilibrium).

The scenario where the stream and lake sites were colonized by a single source population followed by divergence toward a drift-mutation-gene flow equilibrium was evaluated using equation (5) of Chakraborty and Jin (1992). This equation predicts F_{ST} in the absence of gene flow as a function of time (generations), N_e (here the average of $N_{e\ Beerli}$ for the two populations in each lake-stream pairing), the number of subpopulations (here two per pair), and the heterozygosity (average across loci and populations in each pair, from Table 7). Using this approach, we estimated the amount of divergence at equilibrium (F^*_{ST}) and after 10,000 generations ($F^{10,000}_{ST}$) for Misty Lake versus Misty Upper Inlet ($F^*_{ST} = 0.41$; $F^{10,000}_{ST} = 0.21$), Misty Lake versus Misty Lower Inlet ($F^*_{ST} = 0.31$; $F^{10,000}_{ST} = 0.20$), and Misty Lake versus Misty Outlet ($F^*_{ST} = 0.27$; $F^{10,000}_{ST} = 0.20$). If gene flow is present, equilibrium values will be lower and will be reached more quickly. Under this scenario then, the average observed F_{ST}-values of 0.31, 0.14, and 0.03, respectively (Table 8), can be interpreted as divergence from an common source population with almost no gene flow (Misty Upper Inlet), in the presence of slight gene flow (Misty Lower Inlet), and in the presence of strong gene flow (Misty Outlet). Under nonequilibrium conditions in this scenario, our conclusions regarding relative amounts of gene flow would remain the same but our estimates of gene flow and the constraint placed on adaptation would be too high (particularly for the upper inlet). Using a similar analysis for lake versus stream stickleback in the Schleswig-Holstein system, Reusch et al. (2001) also concluded that the observed level of genetic divergence could have arisen postglacially from a common ancestral source.

Periods of geographical isolation have the potential to cause the build-up of divergent gene complexes that result in postzygotic isolation on secondary contact (e.g., Lu and Bernatchez 1998; Gavrilets 2000). If this was the case for the ENA versus TNP lineages, reduced gene flow into the inlet stream could conceivably be the result of preexisting reproductive isolation. This does not appear likely, however, at least for Misty Lake versus Misty Lower Inlet because survival from fertilization to hatching in the laboratory was 93.4% for pure lake fish, 92.9% for pure stream fish, and 96.2% for F_1 hybrids (Lavin and McPhail 1993). Qualitative observations during our own common-garden experiments also suggested no survival disadvantage in hybrids. Instead, any intrinsic reproductive isolation would probably have an

ecological context, which should reflect divergent adaptations rather than historical origins (see Alternative 1 section).

Conclusion

Natural selection causes the adaptive divergence of threespine stickleback in different environments. However, adaptive divergence may in certain situations be constrained by gene flow from elsewhere (see also Bell and Richkind 1981; Bell 1982). Such a constraint could exist when gene flow is high and would also cause a reduction of mean population fitness. However, constraints on adaptation (deviations of mean phenotype from optimal phenotype) could also be caused by low levels of gene flow, if stabilizing selection around alternative adaptive peaks is relatively weak. In this case, the population will appear maladapted but will actually not suffer a severe reduction of population fitness. This appears to be the case in our study because gene flow into the stream population that was least morphologically divergent (outlet) was still fairly low on a proportional basis (m = 0.0027). When the strength of selection varies among sites, relative levels of gene flow may also be influenced by ecologically dependent reproductive isolation. Adaptive divergence will thus reflect a complex interaction between selection and gene flow, with each having the potential to impact the other as well as the amount of adaptive divergence.

We took an integrated approach to investigating factors that influence divergence in lake and stream stickleback. We are continuing to work in this system and foresee several profitable lines of investigation. A more sophisticated breeding experiment would reveal the genetic architecture underlying divergence in adaptive traits. This is important because the nature of nonadditive genetic variation could influence the rate and direction of evolution. A better characterization of environmental variation among the different stream sites would allow a better characterization of variation in the strength of divergent selection. Reciprocal transplant experiments performed in other seasons, in the outlet stream, and using laboratory-reared fish (to control for prior experience) would provide a better indication of how well adapted the different forms are to their home environments. Release-recapture experiments that test for differential survival or reproduction without the confounding effects of emigration would better reveal the strength of selection against lake fish in streams. Examination of genetic and morphological variation along the length of streams would allow cline-based analyses of the effects of gene flow (e.g., Bell and Richkind 1981). Finally, mate-choice experiments would reveal the extent of prezygotic isolation. In short, much work remains to be done before alternative hypotheses can be conclusively discarded.

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