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QUANTIFYING THE CONSTRAINING INFLUENCE OF GENE FLOW ON ADAPTIVE DIVERGENCE IN THE LAKE-STREAM THREESPINE STICKLEBACK SYSTEM

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The constraining effect of gene flow on adaptive divergence is often inferred but rarely quantified. We illustrate ways of doing so using stream populations of threespine stickleback (*Gasterosteus aculeatus*) that experience different levels of gene flow from a parapatric lake population. In the Misty Lake watershed (British Columbia, Canada), the inlet stream population is morphologically divergent from the lake population, and presumably experiences little gene flow from the lake. The outlet stream population, however, shows an intermediate phenotype and may experience more gene flow from the lake. We first used microsatellite data to demonstrate that gene flow from the lake is low into the inlet but high into the outlet, and that gene flow from the lake remains relatively constant with distance along the outlet. We next combined gene flow data with morphological and habitat data to *quantify* the effect of gene flow on morphological divergence. In one approach, we assumed that inlet stickleback manifest well-adapted phenotypic trait values not constrained by gene flow. We then calculated the deviation between the observed and expected phenotypes for a given habitat in the outlet. In a second approach, we parameterized a quantitative genetic model of adaptive divergence. Both approaches suggest a large impact of gene flow, constraining adaptation by 80–86% in the outlet (i.e., only 14–20% of the expected morphological divergence in the absence of gene flow was observed). Such approaches may be useful in other taxa to estimate how important gene flow is in constraining adaptive divergence in nature.

KEY WORDS: **Adaptive divergence, gene flow, isolation-by-distance, microsatellites, migration load, natural selection, threespine stickleback.**

Adaptation in response to spatial variation in the environment is thought to proceed as a balance between diversifying natural selection and homogenizing gene flow (Slatkin 1985a; García-Ramos and Kirkpatrick 1997; Hendry et al. 2001). Although the relative strength of these two forces has been the subject of much debate (Slatkin 1987; Barton 2001), a considerable body of empirical evidence confirms that gene flow *can* constrain adaptive divergence (e.g., Riechert 1993; Sandoval 1994; King and Lawson 1995; Storfer and Sih 1998). However, relatively few studies attempt to *quantify* the magnitude of the constraint imposed by gene

flow on a given population. In this study, we illustrate two potential approaches that can be used to make such inferences in natural populations.

A quantitative assessment of the constraining effects of gene flow on adaptation would be useful for several reasons. First, theoretical work suggests that gene flow might in some circumstances have a positive influence on adaptation (e.g., Holt and Gomulkiewicz 1997; Kawecki 2000; Alleaume-Benharira et al. 2005). The relative importance of positive and negative effects is currently difficult to assess in nature (Lenormand 2002), in part because we still lack a quantitative appraisal of the negative effects alone. Second, it is currently difficult to make predictions regarding the evolutionary consequences of a given increase or decrease in gene flow (Barton 2001). It thus remains difficult for conservation biologists to predict the fitness impacts of translocations and stocking (Storfer 1999). An understanding of such effects will require information on the quantitative effect of gene flow on particular traits, as well as the links between those traits and fitness (Lenormand 2002).

A common approach for detecting the effect of gene flow on adaptation is to compare populations in similar habitats that receive different levels of gene flow from another habitat (Stearns and Sage 1980; Riechert 1993; King and Lawson 1995; Dias and Blondel 1996; Storfer and Sih 1998; Postma and van Noordwijk 2005). This approach also offers an opportunity to quantify the impact of gene flow on phenotypic traits. This next step, however, is rarely taken and these studies have not collected (or reported) the necessary information to do so. One frequently missing piece of information is the quantitative assessment of differences (or similarities) between environments (e.g., Riechert 1993; Postma and van Noordwijk 2005). Another is the frequent lack of quantitative estimates of gene flow, that is, many studies rely on indirect estimates such as geographical distance (e.g., Dias and Blondel 1996). Without estimates of both selection and gene flow, quantitative estimates of the constraint imposed on adaptation are difficult.

Our aim was to illustrate how such an assessment can be made through quantitative estimates of adaptation (based on morphology), gene flow (based on microsatellite genotypic data), and selection (based on habitat data). We specifically examined constraints to adaptation in an outlet stream population of threespine stickleback (*Gasterosteus aculeatus*) hypothesized to receive high levels of gene flow from a parapatric lake population. These inferences were aided by comparison to an inlet stream population in the same watershed, which is hypothesized to receive little gene flow from the lake population.

LAKE AND STREAM THREESPINE STICKLEBACK

Lake and stream stickleback often differ in ways that appear adaptive for their respective environments. Most dramatically, stream fish have deeper bodies and fewer gill rakers than lake fish; differences that have an additive genetic basis (Moodie 1972a,b; Reimchen et al. 1985; Lavin and McPhail 1993; Hendry et al. 2002). With respect to body depth, streamlined (shallow) bodies are better suited for prolonged swimming, which is presumably more important in lakes, whereas robust (deep) bodies are better suited for burst swimming and precise maneuvering, which is presumably more important in streams (Taylor and McPhail 1986; Walker 1997). More numerous gill rakers are better suited for feeding on zooplankton, which predominate in lakes, whereas fewer are better suited for feeding on benthic macro-invertebrates, which predominate in streams (Hagen and Gilbertson 1972; Bentzen and McPhail 1984; Gross and Anderson 1984; Lavin and McPhail 1986). Stream fish also differ from lake fish in several armor characteristics, such as pelvic spines and lateral plates (Moodie 1972a,b; Reimchen et al. 1985; Lavin and McPhail 1993; Hendry et al. 2002), but the direction of lake-stream divergence for these traits is not consistent across systems (Hendry and Taylor 2004).

In the Misty Lake watershed (Fig. 1), lake and inlet stickleback show archetypal lake-stream morphological divergence (Lavin and McPhail 1993; Hendry et al. 2002; Moore and Hendry 2005), and transplant experiments suggest local adaptation to their respective environments (Hendry et al. 2002). Outlet fish, in contrast, show morphological characteristics that are intermediate between lake and inlet fish (Hendry et al. 2002; Moore and Hendry 2005). Outlet fish also show a gradual spatial cline in morphology, from "lake-like" near the lake to "stream-like" farther downstream from the lake (Moore and Hendry 2005). This cline might be driven by a combination of two processes. First, dispersal from the lake into the outlet (and therefore the constraining effects of gene flow) may decrease with increasing distance from the lake. This hypothesis is plausible if stickleback dispersal distances are sufficiently limited. Second, selection for stream-like morphology may increase with increasing distance from the lake. This hypothesis is also plausible given that outlet sites near the lake are more lake-like (deeper and wider) than are outlet sites far from the lake (Moore and Hendry 2005). Data on the spatial variation in gene flow should allow an evaluation of the relative importance of these two processes.

QUANTIFYING THE CONSTRAINT ON ADAPTATION—TWO APPROACHES

Our first approach involved comparing the morphology of two different populations found in similar stream environments, and to relate any observed differences to the extent of gene flow each receives from the same lake population, while also controlling for differences in habitats. In the Misty watershed, the inlet stream population appears well adapted to a benthic browsing foraging mode (Lavin and McPhail 1993; Hendry et al. 2002), and may therefore be reasonably close to its phenotypic optimum. We made this inference for two reasons. First, levels of gene flow

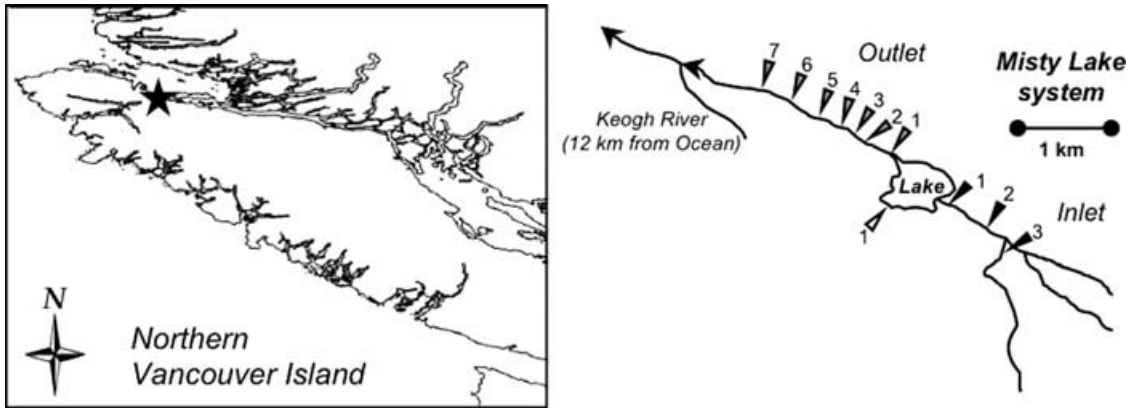

Figure 1. **Sampling locations and site numbers in the Misty Lake system. The star indicates the location of the Misty Lake watershed on Vancouver Island, British Columbia, Canada.**

between the lake and the inlet are so low (mean $F_{ST} = 0.126$; see below) that gene flow appears to have little-to-no effect on adaptation (Hendry et al. 2002). Gene flow is probably restricted by some partial physical barriers (beaver dams, swamps), behavioral differences (lake stickleback move downstream when placed in the inlet, Hendry et al. 2002), selection against migrants (K. Räsänen and A. Hendry, unpubl. data), and partial mating isolation (K. Räsänen and A. Hendry, unpubl. data). Second, phenotypic divergence between the lake and inlet populations in the Misty system is among the greatest previously documented on Vancouver Island (Hendry and Taylor 2004). The only populations that show more extreme phenotypes are those found in even more extreme stream habitats (i.e., shallower and faster flowing waters; D. Berner and A. Hendry, unpubl. data).

Although we use the morphology of inlet stickleback to infer "optimality," we mean this only in the restrictive sense that morphological adaptation in the inlet population is minimally constrained by gene flow from the lake. Moreover, any influence that gene flow does have on inlet sites would make our conclusions about the constraint imposed by gene flow in the outlet (see below) more conservative. It is also possible that other forces (e.g., history, genetic constraints) influence inlet stickleback morphology, but these forces are likely to have a similar influence in the outlet. In short, it seems reasonable to assume that differences in morphology between the inlet and outlet are primarily the result of different levels of gene flow from the lake and/or differences in selective environments. Using the inlet morphology as the expected stream phenotype, we can then determine the extent to which the morphology of outlet fish in a particular stream environment (defined by its habitat features) deviated from the morphology of inlet fish in a similar stream environment.

Our second approach was to parameterize quantitative genetic models of the selection-gene flow balance. Such models might follow an isolation-by-distance (IBD) structure (García-Ramos and Kirkpatrick 1997) or an island-continent structure (Hendry et al. 2001). The type of model most appropriate for our data was determined based on the pattern of gene flow revealed by variation in microsatellites. This second approach is useful because it provided an independent way to estimate the same constraint parameter as that in our first approach. Any differences between the two estimates may therefore be instructive about the utility of the various assumptions made in each.

It is important to recognize that both approaches required numerous simplifying assumptions and we do not consider our specific estimates to be definitive. Instead they provide a heuristic demonstration of how the constraint imposed by gene flow can be *quantified*. If enough data accumulate from studies in other systems, we may eventually be able to make generalizations about the distribution of quantitative effects that gene flow can have on adaptation in natural populations.

Materials and Methods

STUDY SITES, MORPHOLOGY, AND HABITAT DATA

Misty Lake (50°36′32″N, 127°15′46″W) is located 15 km upstream of the Pacific Ocean in the Keogh River system on northeastern Vancouver Island (Fig. 1). Despite this proximity to the ocean, we have never captured anadromous stickleback in the Misty system or in the Keogh River. We collected stickleback from seven sites along the outlet stream, three sites along the inlet stream, and one site in the lake (previous work has shown little variation within the lake; Moore and Hendry 2005). In July 2003 and May–June 2004, we used unbaited minnow traps to collect stickleback from each site (Fig. 1). Samples were collected in both years at four sites: outlets 1 and 4, lake 1, and inlet 3. Thirty individuals per site were sacrificed with an overdose of tricaine methanesulfonate (MS-222) and preserved in 95% ethanol.

Raw morphological data used in the present study were collected by Moore and Hendry (2005). In brief, the following morphological measurements were taken on each fish: body length,

body depth, pelvic spine length, upper jaw length, number of gill rakers, number of lateral plates, and pelvic girdle width. Traits correlated with body size (body depth, pelvic pine length, upper jaw length, and pelvic girdle width) were standardized to a common body size according to Moore and Hendry (2005). Group centroids from discriminant functions were then used as a multivariate composite index of morphological variation (Hendry et al. 2002; Hendry and Taylor 2004; Moore and Hendry 2005). For this analysis (site as the grouping variable), we used two trophic traits (standardized body depth and gill raker number) and two armour traits (standardized pelvic spine length and the number of lateral plates). These traits were chosen because they have a clear adaptive significance and are likely under divergent selection between lakes and streams (see Introduction). The discriminant functions in the present study differed somewhat from those in Moore and Hendry (2005) because some sites were excluded from the present analysis. In addition, we analyzed all 30 collected fish together, whereas Moore and Hendry (2005) analyzed the sexes separately and excluded any fish whose sex was ambiguous. These decisions were made to simplify the present analysis, and they had no impact on our conclusions.

Habitat features were measured between 23 May and 4 June 2004. The specific habitat features were selected to encapsulate those that might influence divergent selection between lakes and streams. That is, more lake-like habitats should have larger open areas (stream width), deeper water, less shading (canopy openness), lower water flow, and fewer rocks. At each site, we established 11 transects evenly spaced every 5–10 m along the stream. This spacing was constant at each site but varied among sites depending on the area from which stickleback were collected. At each transect, we measured the wetted width of the channel (m). At each of three equidistant points across each transect, we recorded water depth (cm), water flow ($m \cdot s^{-1}$), and substrate type (rock, mud, wood, sand, vegetation). When the substrate was a rock, we measured its median diameter (mm). Canopy openness was quantified with a concave spherical densiometer (Lemmon 1957). We then combined all of the habitat features into a single multivariate composite index by using the group centroid of discriminant functions (site as the grouping variable; substrate types other than rocks were given a value of 0).

MICROSATELLITE AMPLIFICATION AND SCREENING

DNA was extracted from the caudal fin tissue of all samples collected in 2003 (30 individuals per site) using a PUREGENE DNA isolation kit (Qiagen, Valencia, CA). These samples were then polymerase chain reaction (PCR) amplified (according to Gow et al. 2006) and screened for length polymorphism at six microsatellite loci (Rico et al. 1993; *Gac*51–52; Taylor 1998; *Gac*4; Peichel et al. 2001; *Stn*216, *Stn*386, *Stn*43, *Stn*254). Analysis of length polymorphism was performed on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA) according to Gow et al. (2006). These specific loci were chosen for the analysis because they have proven reliable and informative in previous work (Taylor and McPhail 2000; Hendry et al. 2002; Hendry and Taylor 2004; Gow et al. 2006).

Two of the microsatellites we used are linked to quantitative trait loci (QTL) in at least some other species pairs of threespine stickleback. *Stn*216 is linked to a plate size modifier in a freshwater-anadromous pair (Colosimo et al. 2004), and *Stn*43 is near the plate morph major locus in a freshwater-anadromous pair and a benthic-limnetic pair (Peichel et al. 2001; Colosimo et al. 2004). It is unlikely that these QTL are under selection in the Misty watershed, given that these traits do not differ across the system. However, we repeated genetic analysis without them to ensure that patterns of differentiation reflect neutral gene flow rather than linkage to selected loci. Lake-outlet comparisons were not changed by the exclusion of the QTL and our conclusions regarding the constraint to gene flow are therefore robust to this reanalysis.

GENETIC DATA ANALYSIS

Each sample was tested for Hardy–Weinberg and linkage disequilibrium using GENEPOP (1000 dememorizations, 100 batches, 1000 iterations per batch; Raymond and Rousset 1995). Statistical significance was evaluated both before and after sequential Bonferroni corrections (Rice 1989). F_{ST} (θ; Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) were calculated between each pair of sites using GENEPOP (Raymond and Rousset 1995). Confidence intervals for pairwise F_{ST} were generated by bootstrap sampling over all loci with FSTAT (ver. 2.9.3; Goudet et al. 1996). The relative suitability of R_{ST} versus F_{ST} was evaluated by determining if allele size significantly contributed to population differentiation (allele permutation test implemented in SPAGeDi ver. 1.2; 20,000 permutations; Hardy and Vekemans 2002).

If stickleback dispersal is spatially restricted in the outlet, then gene flow might follow an IBD pattern. We tested for IBD by using GENEPOP to generate the graphs of pairwise $F_{ST}/(1 - F_{ST})$ against pairwise geographic distance (as recommended by Rousset 1997). Significance levels were determined using Mantel tests as implemented in ISOLDE by GENEPOP (20,000 permutations). These analyses were conducted twice: once for all sites and once after excluding the inlet sites (because they were highly divergent).

Gene flow was estimated using the maximum-likelihood coalescent program MIGRATE (ver. 2.3.1; microsatellite model, threshold value of 10, default values for all other parameters; Beerli and Felsenstein 1999, 2001). This analysis was based on a full migration matrix that allowed gene flow between all possible pairs of sites. For each pair of sites, m was estimated from the bidirectional N_em and N_e estimates as described in Hendry et al.

(2002). The resulting *m* values were analyzed in an IBD fashion. Partial Mantel tests were then used to test for the independent effects of distance and comparison type (i.e., pairs with or without an inlet site).

Finally, we assessed population structure using the program STRUCTURE (ver. 2.1; Pritchard et al. 2000). This program identifies distinct populations by identifying clusters of individuals that minimize linkage and Hardy–Weinberg disequilibria across the dataset. Three independent iterations of $K = 1$–10 and additional single iterations of $K = 12$–25 were performed (for reasons unknown, STRUCTURE was unable to run our entire dataset for values of $K = 11$ and 20). The iterations were performed with 100,000 Markov Chain Monte Carlo (MCMC) repetitions preceded by 100,000 burn-in repetitions, using the admixture and correlated allele frequencies models. The most probable number of clusters (K) was then determined using the ad hoc statistic ΔK proposed by Evanno et al. (2005). Finally, we used the population assignment test implemented in STRUCTURE to classify individuals back to the inferred clusters.

THE EFFECTS OF GENE FLOW

We quantified the constraining effects of gene flow on morphology by using two different and complementary approaches. In the first approach, we calculated the deviation of *observed* phenotypic values in the outlet from those *expected* if gene flow was not constraining adaptation. *Observed* values were specified as the calculated linear relationship between the multivariate morphological index and distance from the lake (Fig. 2A). *Expected* values were determined based on the linear relationship between the multivariate habitat index and distance from the lake (Fig. 2B). Specifically, expected values were calculated by first determining the location in the outlet in which the habitat index was similar to that in the inlet. To account for variation in the inlet, we used two different values of the index to represent inlet habitat: (1) the average across all inlet sites, and (2) the inlet site the most divergent from the lake (i.e., inlet 2). We then assumed that, in the absence of gene flow, phenotypic values at this location in the outlet should be the same as those in the inlet. *Expected* phenotypes at other sites in the outlet were then specified using a linear relationship from lake-like morphological index at the lake (distance 0) through the expected phenotype at the outlet site that had habitat values similar to that in the inlet, as determined above (Fig. 3). This was possible because the habitat index in the outlet varied approximately linearly with distance.

In the second approach, we parameterized quantitative genetic models of the selection-gene flow balance. The most appropriate model structure depends on the spatial pattern of gene flow in the outlet. If we find that gene flow decreases with distance (i.e., significant IBD), a clinal model might be best (e.g., García-Ramos and Kirkpatrick 1997). If we found that gene flow is constant throughout the outlet (i.e., no IBD), a continent-island model may be best (Hendry et al. 2001). Our results unequivocally supported the latter type of structure (see below), and the critical parameter is the overall migration rate (m) between the lake and all outlet sites

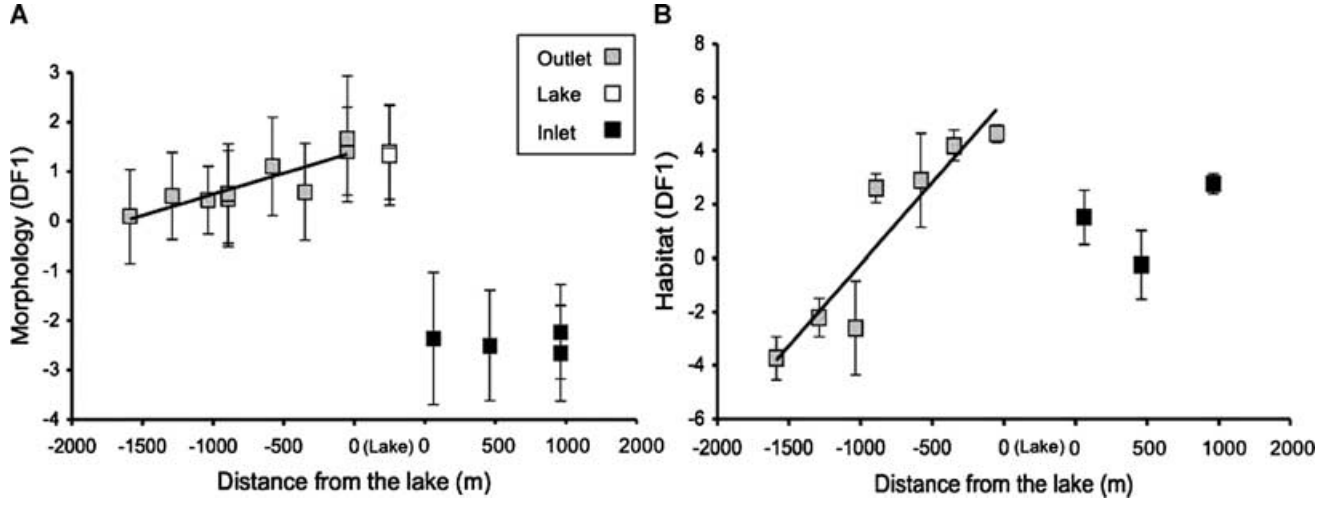

Figure 2. **Morphological and habitat variation in the Misty Lake system. (A) Variation in the multivariate morphological index calculated as the group centroid of the discriminant function at a given site. Sites for which there are two datapoints (outlets 1 and 4, lake 1, inlet 3) were sampled in two different years (2003–2004). (B) Variation in the multivariate index of habitat variation calculated as the group centroid of the discriminant function at a given site. No values are given for the lake because some of the variables measured in the streams have no equivalent for lakes. Negative distances represent distance downstream from the lake in the outlet and positive distances represent distance upstream from the lake in the inlet. The gap between the two zero values does not represent distance in the lake and is present only to facilitate visualization. Lines show significant linear relationships. Error bars show standard deviations in both panels.**

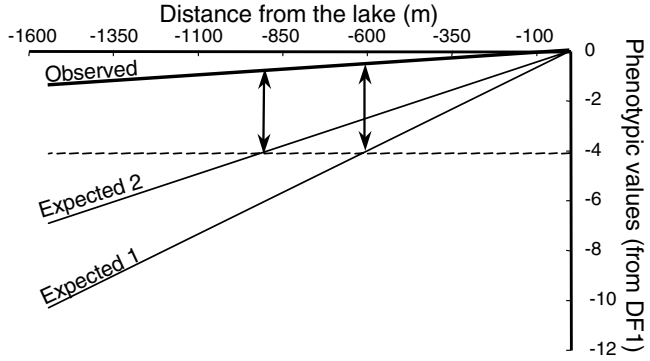

Figure 3. Quantification of the observed (thick line) and expected (thin lines) morphological trait values (group centroids) in the outlet. The dashed line represents the difference between the lake phenotypic value (here set to zero for simplicity of presentation) and average inlet stream phenotypic value. The first estimate of expected phenotype (expected 1) was generated using the average inlet habitat value (group centroid). The second estimate of expected phenotypic value (expected 2) was generated using the most extreme habitat found in the inlet (i.e., inlet 2). The arrows indicate the location in the outlet where habitat values are equivalent to the two estimates of inlet habitat values. At these locations, the outlet phenotypic value should be equivalent to the inlet average (dashed line). The expected relationship between geographical distance and phenotypic value was obtained by passing a line through the origin (lake morphology) and the points where outlet morphology should be equal to inlet morphology. The deviation between the observed and expected phenotypic trait values is attributed to the constraining effect of gene flow on adaptation.

pooled. Because no consensus exists on how best to estimate this parameter from genetic data (Whitlock and McCauley 1999), we used four alternative approaches. First, we calculated $N_e m_{\text{Wright}}$ from F_{ST} by using Wright's (1931) infinite island model ($F_{ST} = 1/[1 + 4N_e m]$). Second, we calculated $N_e m_{\text{Takahata}}$ from F_{ST} by using Takahata's (1983) finite island model and assuming two populations (lake and outlet; $L = 2$) ($F_{ST} = 1/[1 + 4N_e m(L/[L-1])^2]$). Third, we calculated $N_e m_{\text{Slatkin}}$ using Slatkin's (1985b) private allele method as implemented in GENEPOP. Each of these three methods estimates $N_e m$, which we convert to m by dividing $N_e m$ by N_e. N_e was estimated as $H_e/(1 - H_e)4\mu$, where H_e is the expected heterozygosity and μ is the mutation rate (Waples 1991), here assumed to be 10^{-4} (Ellegren 2000). This crude method was used because we did not have the necessary data to estimate N_e using the temporal method, and because the linkage disequilibrium method led to unrealistic estimates given mark–recapture estimates of population size (J.-S. Moore, unpubl. data). Ninety five percent confidence intervals around N_e estimates were calculated using the upper and lower bounds of the 95% confidence intervals of the heterozygosity estimates. Finally, we calculated m_{Beerli} by using the $N_e m$ and N_e estimates from MIGRATE (see Hendry et al. 2002), again assuming the mutation rate to be 10^{-4}. As a comparison, we also generated all estimates of m between the lake and the pooled inlet sites.

Results

MORPHOLOGICAL AND HABITAT DATA

For morphology, the first discriminant function explained 93.2% of the total variation, with the following standardized loadings: gill raker number = 0.567, standardized body depth = −0.646, lateral plate number = −0.160, and standardized pelvic spine length = 0.455. Group centroids from this function differed markedly between the lake and the inlet (Fig. 2A), but showed no trend with distance upstream along the inlet ($r^2 = 0.02$; $P = 0.852$). In contrast, group centroids varied in a linear fashion with distance along the outlet ($b = 0.0009$; $r^2 = 0.77$; $P = 0.002$). Specifically, outlet fish near the lake were lake-like in morphology, whereas those farther from the lake were increasingly inlet-like, although never converging on inlet morphology (Fig. 2A). These results closely parallel those reported by Moore and Hendry (2005) indicating that our methods were consistent between studies.

For habitat, the first discriminant function explained 74.9% of the total variation, with the following standardized loadings: stream width = 0.180, stream depth = 0.340, water flow = −0.128, substrate size = −0.303, and canopy openness = 0.824. In the inlet, no obvious trend in habitat features was evident ($r^2 = 0.22$; $P = 0.689$). In the outlet, however, the habitat became increasingly stream-like (narrower, shallower, and faster flowing water, less opened canopy, and substrate composed of larger rocks) with increasing distance downstream from the lake ($b = 0.0061$; $r^2 = 0.85$; $P = 0.003$). Although a nonlinear model could also have been fit, the linear fit explained a large proportion of the variance and simplified the analysis.

MICROSATELLITE VARIATION, GENE FLOW, AND POPULATION STRUCTURE

Depending on whether one corrects for multiple comparisons, four or five of the six loci showed heterozygote deficits (see Appendix 1, online Supplementary Material). These deficits appear to be the result of population structure within sites (Wahlund effect), rather than screening errors, null alleles, or selection. First, these loci have been used in previous studies in the same laboratory without evidence of screening errors or null alleles (Taylor and McPhail 2000; Hendry et al. 2002; Hendry and Taylor 2004; Gow et al. 2006). Second, although two of the loci are putatively linked to QTL for plate morph, all of the fish in the present study had the same plate morph. Moreover, deficits were mostly observed for loci that were not linked to known QTL. Third, deficits were largely concentrated in the outlet sites, whereas scoring errors or selection might have distributed them more evenly among habitats.

Linkage disequilibrium was not evident between any pair of loci when all sites were pooled ($P > 0.1$). The only pair of loci

that showed significant linkage disequilibrium *within* more than one site (inlets 2 and 3) was *Gac*4 and *Stn*386, and this result was only marginally significant ($P = 0.046$ and $P = 0.049$, before Bonferroni correction). We conclude that none of the loci in our study is physically linked with each other. Pairwise F_{ST} values were highly correlated with pairwise R_{ST} values ($r = 0.96$), and allele size significantly contributed to population differentiation in only one of the 55 possible pairs (results from SPAGEDi). We therefore further consider F_{ST} instead of R_{ST}.

All of the subsequent analyses support the conclusions that (1) inlet stickleback are very genetically distinct from lake and outlet stickleback, (2) lake and outlet stickleback are not genetically distinct, and (3) gene flow is spatially homogeneous throughout the outlet. First, mean pairwise F_{ST} values were higher by an order of magnitude between lake and inlet sites (mean = 0.126) than between lake and outlet sites (mean = 0.012). Moreover, pairwise F_{ST} values between lake and outlet sites were at least as low as those between outlet sites (mean = 0.016). In addition, F_{ST} values for lake-outlet pairs do not increase with increasing distance from the lake, remaining very small even for the most distant sites. Indeed, lake-outlet F_{ST} values had 95% confidence intervals that included zero even for the three farthest outlet sites (outlets 5, 6 and 7; see Appendix 2, online Supplementary Material).

Second, IBD analyses revealed no evidence of spatial restrictions on gene flow in the outlet. For example, pairwise F_{ST} was positively correlated with distance when inlet sites were included (Mantel test; $r = 0.682$; $P = 0.003$; Fig. 4) but not after they were removed (Mantel test; $r = -0.179$; $P = 0.635$; Fig. 4). For m from MIGRATE, a simple Mantel test including all sites failed to identify a relationship between m and distance ($r = 0.041$; $P = 0.652$). A partial Mantel test controlling for the type of comparison (pairs with or without an inlet site) also failed to detect a significant relationship ($r = 0.126$; $P = 0.191$). In contrast, a partial Mantel test controlling for the effect of distance revealed that pairs including at least one inlet site exchanged fewer genes than those not including at least one inlet site ($r = 0.153$; $P = 0.020$).

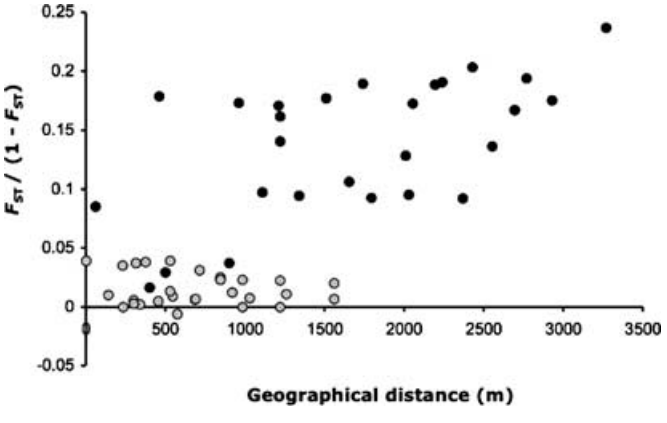

Figure 4. Isolation-by-distance plot based on Rousset's (1997) linearized F_{ST} plotted against pairwise geographical distances. This particular plot is based on an analysis including all sites. Gray-filled circles are pairwise comparisons between lake and outlet sites or between two outlet sites. Black circles are pairwise comparisons between lake and inlet sites or between two inlet sites.

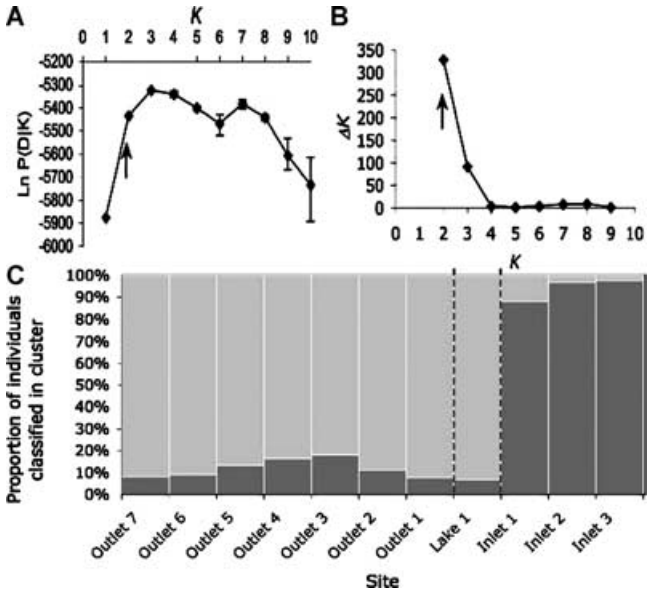

Figure 5. Results of the analysis conducted with STRUCTURE. (A) Log-likelihood profile for the whole dataset, averaged over three independent runs. The error bars represent maximum and minimum log-likelihood values for a given number of putative populations (K). (B) Profile for the ad hoc statistic ΔK (Evanno et al. 2005) plotted against various values of K, suggesting that 2 is the most likely number of clusters. (C) Results of the population assignment tests performed using STRUCTURE. Assuming that $K = 2$, we show for each site the proportion of individuals that are classified to each cluster (again, averaged over three independent runs). The two clusters are shown in two shades of gray.

Third, the analyses in STRUCTURE revealed that inlet stickleback represent a distinct genetic cluster from lake and outlet stickleback, which cluster together (Fig. 5C). The distribution of the ad hoc statistic ΔK (Evanno et al. 2005) was modal at $K = 2$, suggesting the most probable number of genetic clusters, averaged over three iterations, is two (Fig. 5B). The first cluster was comprised almost entirely of inlet individuals whereas the second was comprised almost entirely of the lake and outlet individuals (Fig. 5C). The proportion of individuals classified into the two clusters did not show any consistent spatial pattern in the outlet (Fig. 5C).

THE EFFECTS OF GENE FLOW I—DEVIATION FROM EXPECTED PHENOTYPES

The two multivariate habitat index values used to represent inlet habitat were DF1 = 1.339 (average across all sites) and DF1 = −0.253 (the most extreme site). Based on a linear relationship

between distance and habitat features in the outlet ($b = 0.0061$), these habitat index values occurred at distances of 612 m and 906 m downstream from the lake, respectively. At these locations, in the absence of gene flow, outlet phenotypes should be approximately equal to inlet phenotypes (Fig. 3). Because habitat features beyond these locations in the outlet are even more stream-like (shallower and faster flowing waters) than those in the inlet, outlet morphology beyond these points should also be more stream-like in the absence of gene flow. This seems possible given that we have recently found stream populations in very fast flow/shallow stream sites in other watersheds that show even more extreme "stream-type" morphology than that found in Misty Inlet (D. Berner and A. Hendry, unpubl. data). The proportional deviations between observed and expected values were thus calculated to be 0.80 (using the average inlet habitat value) or 0.86 (using the most extreme inlet habitat value). That is, outlet-lake divergence was only 10–14% as great as expected from habitat features alone. These proportional deviations remained constant throughout the outlet because both lines were linear and had the same intercept (Fig. 3). Note, however, that the *absolute* deviation from the expected phenotype increased with increasing distance downstream from the lake (Fig. 3).

THE EFFECTS OF GENE FLOW II—QUANTITATIVE GENETIC MODEL

All of our analyses confirmed that gene flow was spatially homogeneous throughout the outlet, rather than manifesting a pattern of IBD. We therefore parameterized a quantitative genetic model that considered two patches (lake and outlet) connected by gene flow (Hendry et al. 2001). We used a "migration then selection" model but the results from a "selection then migration" model were very similar. The relevant model is equation (7) in Hendry et al. (2001):

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

where D^* is the equilibrium difference in mean trait value between two patches, D_θ is the difference in the optimum mean trait value, G is the additive genetic variance, P is the phenotypic variance, $\hat{m}$ is the sum of the migration rate in the two directions, and ω is the strength of stabilizing selection.

Using expected heterozygosities averaged over all loci (lake $H_e = 0.610$, outlet $H_e = 0.619$, inlet $H_e = 0.659$), we estimated $N_e = 3910$ (95% CI: 680.66 to ∞) for the lake, $N_e = 4062$ (95% CI: 717.5 to ∞) for the outlet, and $N_e = 4831$ (95% CI: 1071.43 to ∞) for the inlet. From these values, we estimated lake-outlet gene flow as $m_{\text{Wright}} = 0.0116$, $m_{\text{Takahata}} = 0.0058$, and $m_{\text{Slatkin}} = 0.0009$, and lake-inlet gene flow as $m_{\text{Wright}} = 0.0004$, $m_{\text{Takahata}} = 0.0002$, and $m_{\text{Slatkin}} = 0.0003$. These were multiplied by a factor of two to estimate $\hat{m}$ in equation (1). Our final estimates of $\hat{m}$, based on MIGRATE, yielded a lake-outlet estimate of $m_{\text{Beerli}} = 0.0005$ and a lake-inlet estimate of $m_{\text{Beerli}} = 0.0002$. The N_e estimates from MIGRATE were smaller but comparable to those estimated with H_e (Lake, $N_e = 998$, Outlet, $N_e = 2477$; Inlet, $N_e = 3718$). Multiple runs of MIGRATE showed those results to be stable.

The phenotypic variance parameter, $P = 1.04$, was the average variance in the multivariate morphological index for the lake and outlet. Because we do not have estimates of G or ω for Misty Lake, we plotted surfaces of the equilibrium proportional deviation from the optimum (i.e., $1 - D^*/D_\theta$) against the two variables. Note that because P almost equals unity, G approximately equals h^2, the narrow-sense heritability.

Resulting surfaces of expected deviation from optimal phenotypic values (Fig. 6) show that the two approaches only come in line for large values of ω (i.e., weak stabilizing selection) and low heritabilities. This is especially true for m_{Slatkin} and m_{Beerli}. Typical heritabilities for stickleback morphological traits are about $0.3P$ (median value for 33 stickleback morphometric traits in Baumgartner 1995). If we assume this value, the strength of stabilizing selection that would correspond to a 0.8 constraint would be $\omega^2 = 51$ for m_{Wright}, $\omega^2 = 102$ for m_{Takahata}, $\omega^2 = 665$ for m_{Slatkin}, and $\omega^2 = 3999$ for m_{Beerli}. We crudely converted these estimates to γ, the quadratic selection gradient, using the equation $\gamma = -1/\omega^2$ (Arnold et al. 2001). This leads to estimates of $\gamma = -0.0196$ for m_{Wright}, $\gamma = -0.00973$ for m_{Takahata}, $\gamma = -0.0015$ for m_{Slatkin}, and $\gamma = -0.0002$ for m_{Beerli}. These values are in line with those reviewed by Kingsolver et al. (2001), where most estimates of γ are near zero. The two approaches are therefore roughly consistent given biologically realistic parameter values.

Discussion

The *magnitude* by which gene flow constrains adaptation will have profound consequences for the mean fitness of populations (Barton 2001; Lenormand 2002). The magnitude of this effect, however, is rarely quantified in natural populations. Here we illustrate two approaches that can be used to quantitatively estimate this value—and many investigators probably have the necessary data to apply these approaches. First, one can compare the phenotypes of populations in similar habitats that receive different levels of gene flow from another habitat, provided that differences in selective environment are controlled for. Second, one can use gene flow data to help parameterize a quantitative genetic model of the selection-gene flow balance. Using both of these approaches, we showed that gene flow from lake stickleback appeared to have a dramatic constraining effect on morphological adaptation of outlet stickleback in the Misty system.

THE EFFECTS OF GENE FLOW

Our comparison of inlet and outlet morphology suggested that gene flow from the lake could constrain morphological adaptation by about 80%. This estimate is certainly crude but it is likely

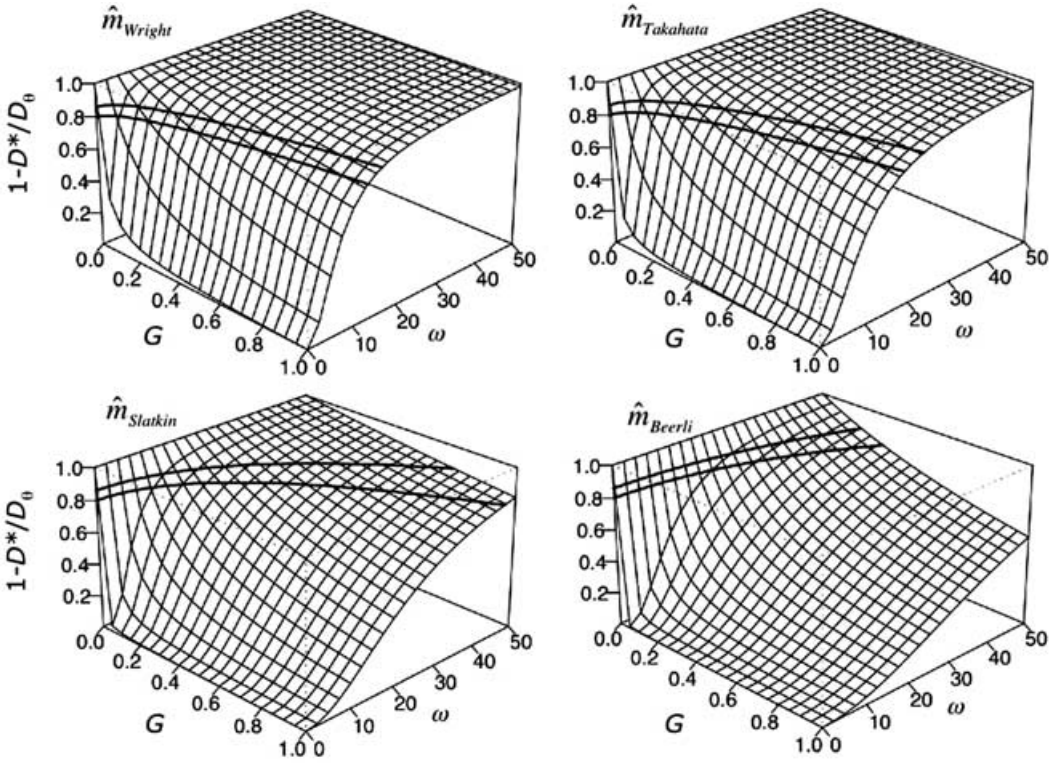

Figure 6. Surfaces of theoretical expectations for the equilibrium proportional deviation between observed and optimal trait values ($1 - D^*/D_\theta$). Parameters include the migration rate, *m*, additive genetic variance, *G*, and the strength of stabilizing selection, ω (eq. 1). Because the observed phenotypic variance, *P*, almost equals unity (1.04), *G* approximately equals narrow-sense heritability, h^2. Migration rate, $\hat{m}$, was calculated using four different methods (detailed in the text): $\hat{m}_{Wright} = 0.0232$; $\hat{m}_{Takahata} = 0.0116$; $\hat{m}_{Slatkin} = 0.0018$; $\hat{m}_{Beerli} = 0.0003$. The thick lines represent the two values of proportional deviation calculated using comparisons with expected phenotypes (i.e., 80% and 86%).

indicative of the real impact of gene flow, because the quantitative genetic model approach yielded similar estimates for biologically realistic parameter values. This suggests a dramatic effect of gene flow on adaptation, but to what extent might this constraint be typical of other natural populations? Comparable data are rarely reported but some studies provide a likely parallel. For example, Hendry and Taylor (2004) found that gene flow explained up to 74.1% of the variation in morphological divergence between lake and stream stickleback populations in other watersheds. Although this is not directly comparable with estimates of the deviation from an optimum, it suggests that the effect of gene flow on stream stickleback morphology is generally important. Several studies have also suggested dramatic effects of gene flow on reducing fitness. For example, Postma and van Noordwijk (2005) stated that local survival in a population receiving little gene flow was "twice as high" as that in a population receiving 43% migrants. Results such as these point to the potential importance of more quantitative estimates of the extent to which gene flow can constrain adaptation in nature.

Although our two approaches were in rough agreement as to the adaptive constraint imposed by gene flow, the quantitative genetic model generally yielded lower estimates for much of the realistic parameter space. There are several possible reasons why the two approaches might come to somewhat different conclusions, with two of them seeming particularly likely. First, the measured habitat variables may not accurately capture the nature of selection on stickleback traits in a given environment. In this study, for example, a given combination of habitat features may select for more lake-like morphology in the outlet than in the inlet (e.g., zooplankton may wash out of the lake). However, the habitat features considered here are known to strongly influence adaptive variation in body shape, gill rakers, and armor traits (Hagen and Gilbertson 1972; Gross and Anderson 1984). Second, the analysis is sensitive to changes in *m* (see Fig. 6), which is difficult to estimate with a high degree of confidence. For instance, three of our estimates of *m* depend on accurate estimates of N_e, and the confidence intervals of our N_e estimates were large. Greater values of N_e would lead to lower values of *m*, which would then decrease the expected constraint on phenotypic divergence. Smaller values of N_e would lead to higher values of *m*, which would then increase the expected constraint on phenotypic divergence, and thus bring the predictions of the genetic model closer to the observed

deviations. The latter scenario seems more plausible given what we know about census population sizes (J.-S. Moore, unpubl. data), and given the lower N_e estimates generated by MIGRATE.

Our estimation method based on linear relationships (Fig. 3) predicted a constant *proportional* deviation from expected phenotypes along the entire outlet. The *absolute* deviation, however, increased with increasing distance from the lake. This suggests that migration load—that is the loss in mean population fitness as a result of immigration of locally maladapted alleles (García-Ramos and Kirkpatrick 1997; Lenormand 2002)—should also increase with increasing distance from the lake. If so, we would expect the population fitness in the outlet to decrease with increasing distance from the lake, which might ultimately limit the species' range in the outlet (sensu Kirkpatrick and Barton 1997; Ronce and Kirkpatrick 2001). Interestingly, we have found that population densities decrease along the outlet, with stickleback becoming exceedingly difficult to find downstream of site 7 (see Fig. 1) (J.-S. Moore, unpubl. data). Outlet stickleback in the Misty watershed may therefore be an interesting system for examining the effects of gene flow on range limits over a small geographical scale.

MORPHOLOGICAL CLINES DESPITE HIGH GENE FLOW: THE ROLE OF NATURAL SELECTION

Because they are unidimensional, stream habitats are often characterized by an IBD pattern of genetic differentiation (e.g., Costello et al. 2003; Crispo et al. 2006). This was not the case for stickleback in the Misty outlet, where gene flow was relatively high and spatially homogeneous. Perhaps this is due to the small spatial scale (the farthest outlet site was only ~1.6 km away from the lake). Indeed, this distance seems easily covered over evolutionary time scales, considering that some Misty outlet stickleback can move over 100 m in only two weeks (J.-S. Moore, unpubl. data), and that other stream stickleback have been found to move up to 1.8 km over one year (D. Bolnick, pers. comm.). However, we also found a large genetic differentiation in the inlet over similar spatial scales. Moreover, the outlet stream contains many beaver dams and fast, shallow riffles that should make movement over large distances more difficult than in sites directly adjacent to the lake (pers. obs.). The lack of spatial restriction to gene flow in the outlet is thus intriguing and has implications for the interpretation of our results.

When one habitat type abuts another, gradual clines in morphology are often taken as evidence for a constraining effect of spatially restricted gene flow (e.g., Bell and Richkind 1981; Dias and Blondel 1996; Moore and Hendry 2005). In the case of Misty outlet stickleback, however, the gradual cline in morphology has arisen even without spatially restricted gene flow. The cline instead appears to reflect spatially varying habitat features coupled with spatially homogeneous gene flow. Several factors may explain the maintenance of a morphological cline in the absence of spatially restricted gene flow. One possibility is phenotypic plasticity in response to local habitat features—but the traits we examined clearly have a genetic basis in this and other stickleback populations (Baumgartner 1995; Peichel et al. 2001; Hendry et al. 2002). A second is that selection may regenerate the cline each generation without leading to any evolutionary response—but this seems unlikely given the magnitude of phenotypic divergence along the outlet (Fig. 2A). A third is that stickleback actively move to areas of the outlet that better suit their morphology (i.e., habitat selection)—but we have found no evidence for phenotype-biased dispersal (J.-S. Moore, unpubl. data). A fourth possibility is that gene flow, even though spatially homogeneous, may still be low enough that some adaptive divergence can take place. This does seem possible given that partial adaptive divergence is possible even at very high gene flow (Hendry et al. 2001). A final possibility is differential gene transfer, that is, selection is able to maintain differentiation at selected loci despite much lower differentiation at unlinked neutral loci (Wu 2001). Perhaps this is why some divergence in morphology was observed despite minimal divergence at neutral microsatellite loci. These various possibilities provide interesting alternatives to test in future work.

Conclusion

We quantitatively estimated the impact of gene flow on adaptive divergence. This analysis was made possible by combining information on dispersal potential (distance), gene flow (microsatellites), selection (habitat features), and adaptation (morphology). We found that gene flow was responsible for a proportional deviation between the observed and expected phenotypes of approximately 80%. The impact of this deviation on mean population fitness will depend on the strength of the relationship between the measured traits and fitness. Interestingly, a cline in selection coupled with spatially homogeneous gene flow dictated that the absolute amount of the deviation from expected phenotypes increased with increasing distance from the lake. Migration load should therefore increase with distance from the lake, which may explain the coincident decline in population density with distance. Perhaps the Misty Lake system provides a small-scale demonstration that gene flow can limit species ranges.

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Appendix 1.
Appendix 2.

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