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When maladaptive gene flow does not increase selection

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Populations receiving high maladaptive gene flow are expected to experience strong directional selection—because gene flow pulls mean phenotypes away from local fitness peaks. We tested this prediction by means of a large and replicated mark-recapture study of threespine stickleback (*Gasterosteus aculeatus*) in two stream populations. One of the populations (outlet) experiences high gene flow from the lake population and its morphology is correspondingly poorly adapted. The other population (inlet) experiences very low gene flow from the lake population and its morphology is correspondingly well adapted. Contrary to the above prediction, selection was not stronger in the outlet than in the inlet, a result that forced us to consider potential reasons for why maladaptive gene flow might not increase selection. Of particular interest, we show by means of a simple population genetic model that maladaptive gene flow can—under reasonable conditions—*decrease* the strength of directional selection. This outcome occurs when immigrants decrease mean fitness in the resident population, which decreases the strength of selection against maladapted phenotypes. We argue that this previously unrecognized effect of gene flow deserves further attention in theoretical and empirical studies.

KEY WORDS: **Adaptation, fitness, gene flow, models/simulations, natural, selection.**

Selection is the primary driver of the evolution of functional traits in natural populations (Darwin 1859; Endler 1986; Schluter 2000; Bell 2008). It is therefore disconcerting that empirical studies of presumed adaptive traits often fail to find statistical support for noteworthy selection (Kingsolver et al. 2001; Hersch and Phillips 2004). The likely reason for this outcome is that selection should be weak in populations that are well adapted to their local environments because such populations should harbor little maladaptive variation: that is, "selection erases its traces" (Haller and Hendry 2014). Of course, some empirical studies do document strong selection (Endler 1986; Kingsolver et al. 2001), which thus implies at least some maladaptation to local environments. One suggested cause of strong selection is genetic constraints that prevent—or severely limit—adaptive responses to selection (Blows and Hoffmann 2005; Hansen 2006; Hansen and Houle 2008). Another suggested cause is environmental change that frequently shifts the adaptive peak, such as through abrupt disturbances, gradual changes (e.g., climate warming), or stochastic environmental noise (Yoshimura and Jansen 1996; Schlaepfer et al. 2002; Both et al. 2006). A final suggested cause of strong selection occurs when maladaptive gene flow holds populations short of their adaptive peaks and thereby maintains directional selection (e.g., Haldane 1930; Endler 1977; Slatkin 1985; Burt 1995; Hendry et al. 2001; Lenormand 2002; Kawecki and Ebert 2004). This last cause, which some theory suggests might not always obtain (see Discussion/Appendix), was the focus of our study.

The expectation that maladaptive gene flow should increase the strength of directional selection has been tested in only a few empirical systems. In one such test, Bolnick and Nosil (2007) showed that populations of *Timema* walking sticks on two host plant species were under stronger selection for crypsis when they were less well adapted as a result of high gene flow from *Timema* on other host plants. In another test, Bolnick et al. (2008) showed that threespine stickleback (*Gasterosteus aculeatus*) in the

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shallow basin of a lake were poorly adapted for the local benthic habitat owing to high gene flow from the adjacent deep basin of the lake, thus maintaining strong selection for benthic-adapted traits in the shallow basin. In the present study, we undertook a new test of the hypothesis that high maladaptive gene flow increases directional selection. Importantly, neither prezygotic barriers (Raeymaekers et al. 2009; Räsänen et. al 2012) nor postzygotic barriers (Lavin and McPhail 1993; Hendry et al. 2002) that would prevent gene flow have been found for our study system.

Our work focuses on threespine stickleback from the Misty lake system in British Columbia, Canada. This system is well suited for our study owing to the contrast between its two stream populations: inlet and outlet. Specifically, the inlet and outlet streams have similar habitats for much of their lengths and yet the phenotypes of their resident stickleback are very different (Hendry et al. 2002; Moore and Hendry 2005; Moore et al. 2007; Fig. 1). Inlet stream stickleback possess a wide array of morphological, behavioral, physiological, and life-history traits that differ dramatically from those of stickleback in the lake just downstream (Lavin and McPhail 1993; Hendry et al. 2002; Delcourt et al. 2008; Sharpe et al. 2008; Raeymaekers et al. 2009; Berner et al. 2011; Hendry et al. 2011; Baker et al. 2013). Outlet stickleback, by contrast, are very similar to lake stickleback in all of the same characters for which inlet stickleback differ so dramatically (Hendry et al. 2002; Moore et al. 2007; Berner et al. 2008; Sharpe et al. 2008; Baker et al. 2013).

The above patterns of divergence are closely associated with gene flow, which is very low from the lake into the inlet and very high from the lake into the outlet (Hendry et al. 2002; Moore et al. 2007). Very high gene flow in the latter case is not surprising because (1) lake stickleback tend to be displaced downstream when placed in flowing water (Hendry et al. 2002), (2) no barrier exists between the lake and its initially slow-moving outlet, and (3) freshwater stickleback in this area do not show intrinsic genetic incompatibilities (Lavin and McPhail 1993; Raeymaekers et al. 2010; Berner et al. 2011) or strong mating isolation (Räsänen et al. 2012; Räsänen and Hendry 2014). The lake population is likely little influenced by gene flow from the streams because it is much larger, inlet fish move upstream, and outlet fish move downstream. These differential patterns of gene flow, coupled with the realization that adaptive phenotypes should be similar between the inlet and most of the outlet (Fig. 1), have generated the strong inference that gene flow from lake stickleback has strongly constrained adaptation in outlet stickleback but not inlet stickleback (Hendry et al. 2002; Moore and Hendry 2005; Moore et al. 2007; Berner et al. 2009; Roesti et al. 2012). Following traditional theory, we therefore predict that directional selection toward a stream-like phenotype should be stronger in outlet stickleback than in inlet stickleback at Misty lake (Moore and Hendry 2009).

Several previous studies have measured selection in wild stickleback (Hagen and Gilbertson 1973; Reimchen and Nosil 2002, 2004; Bolnick and Nosil 2007; Gow et al. 2007; Bolnick and Lau 2008; Kitano et al. 2008; Marchinko et al. 2014), yet none of these studies used individually marked, free-living stickleback. We implemented this approach by means of a mark-recapture study designed to minimize concerns that can bedevil attempts to measure selection. First, we used a clear and important fitness component (Kingsolver et al. 2001; Kingsolver and Diamond 2011): the survival of individual fish. Second, we monitored selection over long time periods (Hoekstra et al. 2001) and across multiple life stages (Schluter et al. 1991): survival over entire summers and entire winters. Third, we temporally replicated our samples (Siepielski et al. 2009, 2011; Morrissey and Hadfield 2012; Haller and Hendry 2014): survival over two summers and two winters. Fourth, we used large sample sizes (Kingsolver et al. 2001; Hersch and Phillips 2004; Haller and Hendry 2014): an average of more than 400 individuals per site and season. Of course, we would ideally have also measured other fitness components (e.g., reproductive success), monitored other life stages (juveniles), had even more replication (more years), and had even larger sample sizes. Moreover, because we studied open populations in the wild, our mark-recapture methods capture *apparent* survival. Despite these imperfections, however, our study represents a particularly robust attempt to measure and compare selection in natural populations.

We start by analyzing a single trait with particularly clear a priori expectations. Body depth shows strong genetically based divergence between Misty lake and inlet stickleback (Lavin and McPhail 1993; McPhail 1994; Hendry et al. 2002; Berner et al. 2011; Hendry et al. 2011). This difference is likely adaptive given that it is similar to that seen in many other lake-stream stickleback (Hendry and Taylor 2004; Kaeuffer et al. 2012; Hendry et al. 2013; Lucek et al. 2013; Ravinet et al. 2013) and that it has a clear functional interpretation (McPhail 1994; Walker 1997; Hendry et al. 2011; McGee et al. 2013). Specifically, fish with shallower bodies are favored in lakes owing to their free-swimming limnetic zooplanktivore lifestyle. By contrast, fish with deep bodies are favored in streams owing to their increased maneuverability in complex environments (Walker 1997; Hendry et al. 2011) and their benthic foraging behavior (McGee et al. 2013). Yet, as noted above, Misty outlet stickleback do not show the deep bodies expected of stream fish, but rather manifest the shallow bodies typical of Misty lake stickleback from further upstream (Fig. 1), seemingly as a result of high gene flow (Hendry et al. 2002; Moore et al. 2007). The shallow bodies of outlet stickleback are likely maladaptive because stickleback throughout most of the outlet, including at our experimental site, (1) experience typically complex stream habitats (Moore and Hendry 2005; Moore et al.

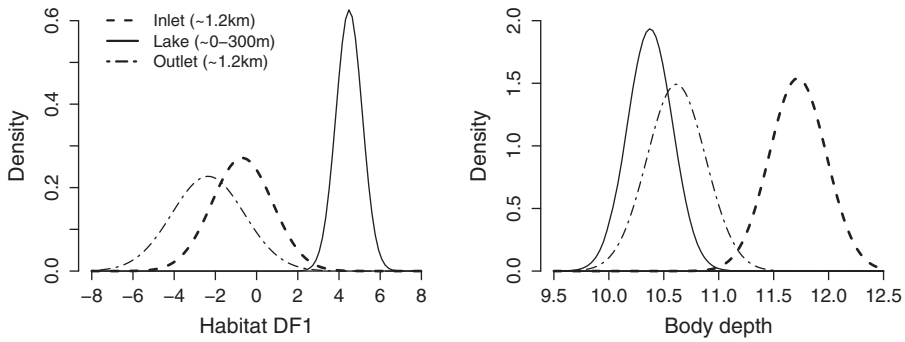

Figure 1. **Distinction and overlap in regard to habitat and phenotype (e.g., body depth) between inlet, outlet, and lake sites at Misty lake. Left panel: distribution of multivariate habitat index scores for a given site are based on water depth (cm), water flow (m/s), substrate type (rock, mud, vegetation, sand), and canopy cover. Data are taken from Moore et al. (2007). The legend depicts the distance of each site from the lake and we here use "lake" and "upper outlet" equivalently because some habitat variables measured in the streams have no equivalent for lakes. Right panel: distribution of body depth variable for the respective sites. Data are taken from Hendry et al. (2002) and are comparable to our inlet and outlet measurements in 2010–2012 (see main text).**

2007) and (2) have benthic diets as opposed to the limnetic diets of lake fish (Berner et al. 2008, 2009). Thus, the clear prediction is that directional selection for deeper bodies should be stronger in the outlet than in the inlet.

Body depth is only one of the many ways in which Misty lake and inlet stickleback differ morphologically whereas Misty lake and outlet stickleback do not (Lavin and McPhail 1993; McPhail 1994; Hendry et al. 2002; Berner et al. 2008, 2009; Kaueffer et al. 2012). We therefore next analyze multivariate directional selection based on a combination of external features that we could reliably quantify on photographs of individually marked fish prior to their release. For this second analysis, we present total selection metrics (see Methods) rather than detailed trait-by-trait coefficients, as is appropriate for studies interested in the total strength of selection on morphology (Schluter 1988; Thorpe et al. 2005). We do not formally assess nonlinear selection in the present article because the predictions based on gene flow are specific to directional selection.

Methods

FIELD WORK AND CAPTURE–RECAPTURE MODELING

The experimental sites were in the upper inlet (~1.1 km upstream of the lake, 50° 36' 07.56'' N, 127° 15' 2.16'' W, hereafter inlet, Fig. S1) and the lower outlet (~1.2 km downstream of the lake, 50° 36' 48.09'' N, 127° 16' 32.01'' W, hereafter outlet, Fig. S1). These specific sites were chosen for their similar environments with respect to abiotic factors (water flow, water depth, stream width, canopy cover, and substrate type, Fig. 1) and biotic factors (diets). At each site, we carried out mark-recapture experiments across five consecutive field seasons: Spring 2010 (May 29–June 1 and June 13–14), Fall 2010 (September 9–15), Spring 2011 (June 1–14), Fall 2011 (August 22–September 1), and Spring 2012 (May 11–June 2). The experiment thus generated mark-recapture data for four selection intervals (S1 = Summer 2010, W1 = Winter 2011, S2 = Summer 2011, and W2 = Winter 2012), with each interval between two consecutive sampling occasions being a distinct selection interval impacting trait distributions. In each season at each site, the fish were collected with unbaited minnow traps. To maximize sampling effectiveness, approximately 90 traps were haphazardly placed throughout the entire sampling area (including upstream and downstream of the area where stickleback were marked and released) and over multiple (4–6) days. Sampling was restricted to stream sites because large population sizes and extensive movement made comparable mark-recapture efforts in the lake unfeasible.

Collected fish underwent a standardized measurement protocol that included weighing (precision to the nearest 0.01 g), digital photographing (left-side-up, Fig. S2) for landmark analysis (details below), and individual marking with unique subcutaneous implant tags (VIalpha tags, Shaw Island, WA). Tags were placed under the skin in the caudal region with a sharp needle injector, a procedure that we previously confirmed through pilot experiments was associated with low mortality and high tag retention (S. Muttalib, unpubl. data). The fish were not anesthetized to minimize handling time. Only fish ≥0.65 g were included in the study because smaller fish were more likely to be harmed by the tagging procedure (S. Muttalib, unpubl. data). Any captured fish that had been tagged and processed within the same sampling season was immediately released back into its capture location. Any captured fish that had been tagged and processed in a previous sampling season had its tag identity recorded and was re-weighed and re-photographed. Following the above procedures, and after 1–2 hours of recovery, the fish were released at their site of capture.

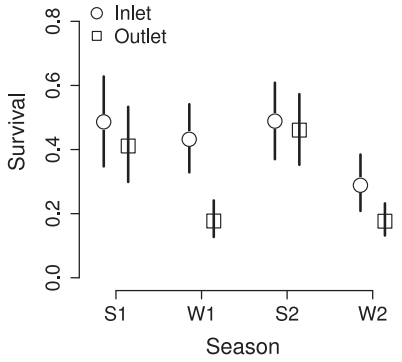

Figure 2. Survival probabilities calculated from CJS mark-recapture models (see Methods) for all four intervals (S1-W2) in the inlet and outlet.

We obtained mark-recapture data for a total of 3940 individual fish across the four selection intervals: Summer 2010 (inlet: $n = 342$, outlet: $n = 464$), Winter 2011 (inlet: $n = 401$, outlet: $n = 258$), Summer 2011 (inlet: $n = 525$, outlet: $n = 950$), and Winter 2012 (inlet: $n = 498$, outlet: $n = 502$). With these data, we used the software MARK (White and Burnham 1999) together with the RMark interface (Laake 2013) to estimate survival and recapture probabilities based on Cormack-Jolly-Seber (CJS) mark-recapture models with maximum likelihood estimation. We tested for overdispersion of our models based on variance inflation ($\hat{c}$) and goodness of fit was then evaluated based on ($\hat{c}$-adjusted) AICc scores according to whether survival probabilities (φ) and/or recapture probabilities (p) varied with time ($\sim t$), sampling site ($\sim s$), their interaction term ($\sim t \times s$), or were constant through time (~ 1).

MORPHOLOGICAL MEASUREMENTS

Digital photographs were used to quantify univariate traits and geometric morphometric shape variables. The traits and landmarks were chosen based on their reliable visibility in photographs and on previous knowledge suggesting their adaptive significance (e.g., Sharpe et al. 2008; Berner et al. 2009; Kaeuffer et al. 2012). The specific univariate measurements were total body length (tbl), jaw length (jl), eye width (ew), pectoral fin width (pfw), pectoral fin length (pfl), pelvic spine length (psl), caudal fin length (cfl), and body depth (bd). These traits were measured in the software tpsDig (version 2.10, Rohlf 2006) as the Euclidean distance between points based on a scale factor. The geometric morphometric shape variables were based on 10 true landmarks that were scored from characteristic shape points on the fish outline and three "semilandmarks," the latter were scored based on a grid superimposed onto each photograph (Fig. S2). The "semilandmarks" were intended to capture additional shape variation along the outline in addition to the true landmarks (Zelditch 2012). The set of 13 (semi)landmarks was analyzed in tpsRelw (version 1.42,

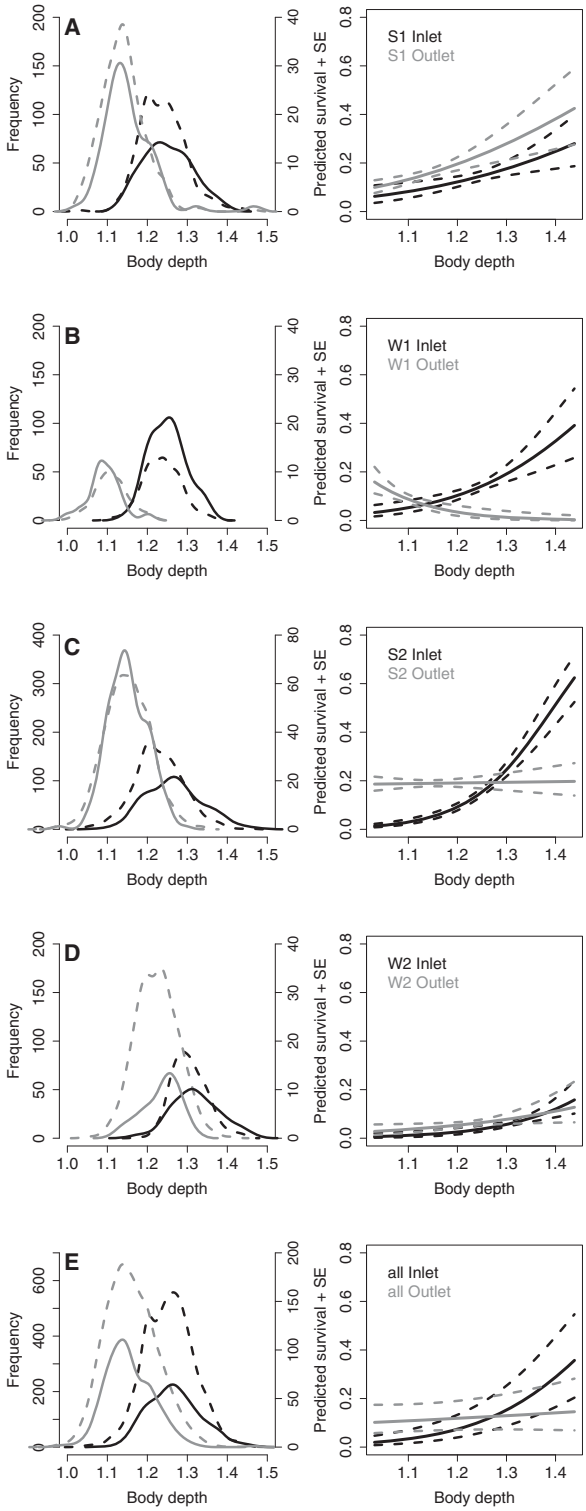

Figure 3. Left panel: frequency distributions of body depth in the inlet and outlet populations across the four selection intervals (A–D) and for all intervals combined (E). Solid lines depict only survivors, dashed lines depict all fish from the respective interval (note the two differently scaled y-axes depicting frequency: left for the outlet, right for the inlet). Right panel: particular fitness curves predicted from the respective binomial GLM(M)s for fitness on body depth (dashed lines show standard errors).

Rohlf 2005) to compute centroid sizes and consensus landmark configurations for particular combinations of site and interval (see below). Consensus configurations were then used to calculate the weight matrix that summarizes shape variation and can be used to extract the relative warps (principal components of the weight matrix).

All univariate measurements and relative warps were standardized to a common body size (Elliott et al. 1995, Lleonart et al. 2000). Total body length (tbl) was used as the body size measure for univariate traits and centroid size was used for the relative warps. Standardizations followed the allometric approach (Lleonart et al. 2000): $M_S = M_0\ (L_S / L_0)^b$, where M_S is the standardized trait measurement, M_0 is the unstandardized trait measurement, L_S is the overall mean body size of all fish in a given analysis, and L_0 is the body size of the individual. The exponent b was calculated as the common within-group slope (Reist 1986) from a linear mixed-effect model regressing $\log_{10}(M_0)$ on $\log_{10}(L_0)$ with the starting (preselection) samples (Summer 2010, Winter 2011, Summer 2011, Winter 2012) as the random factor.

Before selection analyses (see below), we compared the morphology of inlet and outlet stickleback. For this, univariate measurements were standardized to the overall mean body length (50.79 mm) across all seasons using the above approach, and were then compared across all seasons with ANOVAs and variance ratio tests to test for differences in means and variances between the sites. Multivariate differences in morphology were tested with MANOVAs for combined univariate (body-length-standardized) measurements and for all (centroid-size-standardized) relative warps. In addition, we used linear discriminant functions to determine back-classification of individuals to their collection site based on total morphology (i.e., all univariate measurements and all relative warps, separately).

ESTIMATING SELECTION

For size-standardized body depth, we used logistic regression models including fitness (1: survived, 0: dead; note that this measure captures apparent survival only) as the response variable to estimate (1) the directional selection coefficient (β) for the predictor body depth alone, and (2) the partial selection coefficient (β_{part}) for the predictor body depth in models that included all (size-standardized) univariate measurements as predictors. For the selection analyses, the entire recapture history of each individual was taken into account: for example, a capture history of 1/0/1 (caught/not caught/caught) would translate into a response variable of 1/1/1 (alive/alive/alive). To estimate site- and interval-specific selection, separate models were analyzed for each of the eight interval/site combinations. To simultaneously estimate overall selection across the sampling intervals, separate models were analyzed for the two sites while incorporating sampling interval as the random structure. Coefficients from these logistic models were then converted to their linear equivalents ($\beta_{(avg.grad)}$) following Janzen and Stern (1998). Selection coefficients were further converted to mean-standardized selection gradients (β_{mean} and $\beta_{mean,\ part.}$) and variance-standardized selection gradients (β_{sd} and $\beta_{sd,\ part.}$) by multiplying $\beta_{(avg.grad)}$ by the respective trait mean or standard deviation (Hereford et al. 2004; Matsumura et al. 2012). Standard deviations for the logistic regression coefficients from binomial GLMs and GLMMs were obtained through $n = 1000$ model simulations (Gelman and Hill 2006). Differences in directional selection on body depth between the inlet and the outlet for all four intervals and across all intervals were then tested with binomial GLM(M)s that included a body depth $\times$ site interaction. Finally, we compared our standardized linear gradients to the data reported in meta-analyses of directional selection in a variety of natural systems (Kingsolver et al. 2001). Whether or not our analyses used standardized or unstandardized coefficients did not change our interpretation and so we report only the standardized coefficients (i.e., the respective β_{sd} and β_{mean}).

For total directional selection on all measured traits, we used two metrics suggested by previous authors (Schluter 1988; Thorpe et al. 2005), plus an additional metric based on the explained variance in fitness. In each case, analyses were performed separately for univariate traits, relative warps, and (for comparison) body depth—all size-standardized as described above. The first metric was the coefficient of variation in predicted fitness (Schluter 1988) from the above models ($CoV_{predicted}$ and $CoV_{predicted,\ partial}$). This metric can be thought of as the absolute amount of variation in fitness explainable by the measured traits, which will depend on the explanatory power of the traits and the total variance in fitness. Unlike Schluter (1988), we here consider only directional selection, which is the specific form of selection related to our question (see Introduction). The second metric was the Mahalanobis distance in trait space (Thorpe et al. 2005) between fish that survived and fish that died during a particular interval and across all intervals. This metric can be thought of as a multivariate selection intensity that takes into account the within-group covariances. The third metric was the proportion of variance in fitness explained by the traits in the model, a relative measure that standardizes for variance in fitness. For the generalized linear models (i.e., interval-specific models), this last metric was calculated as the Nagelkerke pseudo-R^2 of the respective logistic model. For the generalized linear mixed models (i.e., models combining all intervals), R^2_{GLMM} estimates were obtained following the logic of Nakagawa and Schielzeth (2013). Both pseudo-R^2 and R^2_{GLMM} scale between 0 (no explanatory power) and 1 (full fit). Confidence intervals for $CoV_{predicted}$ and $CoV_{predicted,\ partial}$ were based on noncentral t-distributions and standard deviations for pseudo-R^2, R^2_{GLMM}, and Mahalanobis distances were obtained through model bootstrapping

($n = 5000$). All analyses were conducted in R (R Development Core Team).

Results

SEASONAL SURVIVAL AND RECAPTURE PROBABILITIES

During the four intervals (S1, W1, S2, and W2), we made a total of 210 recaptures from the 1766 tagged inlet fish (range: 6.2–14.5%, Σ: 11.9% recaught) and a total of 305 recaptures of the 2174 tagged outlet fish (range: 6.1–18.9%, Σ: 16.3% recaught). Recapture success was generally lower after a winter interval ($\text{mean}_{\text{Inlet}}$: 9.5%, $\text{mean}_{\text{Outlet}}$: 6.8%) than after a summer interval ($\text{mean}_{\text{Inlet}}$: 14.4%, $\text{mean}_{\text{Outlet}}$: 17.3%). The CJS models for survival (φ) and recapture probability (p) across seasons (S1–W2) and sites (inlet vs. outlet) were evaluated based on the goodness of fit to our data using AICc scores (see Table S1 for a summary of candidate models). Here, the best fitting model (lowest AICc; Table S1) for survival and recapture probabilities in the outlet retained a time × site interaction term for survival, φ ($\sim t \times s$), and a site-varying term for recapture probability, p ($\sim s$). Overall, recapture probabilities were lower in the inlet (0.23 ± 0.03 SE) than in the outlet (0.36 ± 0.04 SE), with these recapture rates being comparable to estimates from similar studies of other wild fish species (Crespin et al. 2002; Carlson and Letcher 2003; Lynch and Mensinger 2013). Survival probabilities during summer (S1 and S2) did not differ significantly between sites, but survival probabilities during winter (W1 and W2) were significantly lower in the outlet, in particular in W1 (Fig. 2), indicating exacerbated apparent winter mortality for outlet fish.

DIVERGENCE IN MORPHOLOGY

Inlet and outlet stickleback overlapped considerably in total body length but the average was smaller ($F = 680.3$, $\text{df} = 1$, $P < 0.001$) in the inlet (47.50 mm) than in the outlet (53.46 mm,). Following allometric standardization to a common body length (50.79 mm), inlet and outlet stickleback differed in mean values for all traits except caudal fin length. Specifically, outlet fish had wider pectoral fins and longer pelvic spines than did inlet fish, whereas inlet fish had longer jaws, wider eyes, longer pectoral fins, and deeper bodies than did outlet fish (Table 1). Of particular note, these differences in pelvic spine length and body depth correspond to those documented for wild fish in previous studies (Lavin and McPhail 1993; Hendry et al. 2002) and are known to be genetically based in the Misty system (Sharpe et al. 2008; Berner et al. 2011). Moreover, inlet fish showed consistently lower variance in univariate traits compared to outlet fish (variance ratio test, Table 1). Inlet and outlet fish also differed significantly in multivariate analyses of univariate traits (Pillai's trace = 0.471, $P = 2.2 \times 10^{-16}$, with the highest canonical coefficient for body depth) and geometric morphometrics (Pillai's trace = 0.716, $P = 2.2 \times 10^{-16}$), again confirming previous analyses (Sharpe et al. 2008; Berner et al. 2011; Hendry et al. 2011). Not surprisingly given these many differences, discriminant functions correctly assigned inlet fish to their home population in 80.01% of cases based on univariate traits and in 93.1% of cases based on geometric morphometrics (Fig. S3). The corresponding assignment success for outlet fish was 85.92% (univariate) and 95.5% (geometric morphometrics).

SELECTION ON BODY DEPTH

Selection on body depth differed between inlet and outlet stickleback in two of the four intervals and across all intervals (Figs. 3, 4; Tables 2, S2). Inlet stickleback were under positive directional selection ($\beta_{\text{linear}} > 0$) for deeper bodies across all intervals combined ($\beta_{\text{all}} = 0.83$, $P = 1.4 \times 10^{-9}$) and within three of the four intervals ($\beta_{S1} = 0.53$, $P = 0.10$; $\beta_{W1} = 0.83$, $P = 0.02$; $\beta_{S2} = 1.35$, $P = 4.8 \times 10^{-8}$; $\beta_{W2} = 0.48$, $P = 0.03$). By contrast, the outlet population was not under selection with respect to body depth across all intervals combined ($\beta_{\text{all}} = 0.11$, $P = 0.37$) nor in three of the four specific intervals ($\beta_{S1} = 0.61$, $P = 0.04$; $\beta_{W1} = -0.76$, $P = 0.07$; $\beta_{S2} = 0.03$, $P = 0.90$; $\beta_{W2} = 0.22$, $P = 0.25$). The strongest positive selection was detected for the inlet fish in summer 2012 (S2) and the biggest difference in body depth selection between inlet and outlet fish occurred in Winter 2011 (W1) (Figs. 3, 4, Table S2).

Other measures of selection intensity yielded similar conclusions: if anything, stronger selection in the inlet than in the outlet. In particular, the explanatory power of body depth (as a single trait, or in combination with all univariate traits) based on the variance in predicted fitness ($CoV_{predicted}$ and $CoV_{predicted,\ partial}$) was higher for inlet than outlet fish in two of the four intervals (S1 and W2) and across all intervals combined (nonoverlapping 95% confidence intervals in Fig. 5, Table S2). Similarly, the relative proportion of fitness variance explained by body depth (pseudo-R^2 and R^2_{GLMM}) was higher in the inlet for intervals S1 and W2, and across all intervals combined (Fig. 5, Table S2).

SELECTION ON OVERALL MORPHOLOGY

Relative to the above results for body depth, results combining multiple traits were more variable. Based on univariate traits, $CoV_{predicted}$ was higher in the inlet than in the outlet in S2, higher in the outlet than in the inlet in W1 and W2, and not different between the sites in S1. However, total selection across all four intervals was stronger in the inlet than in the outlet (Fig. 5, Table S2). Similarly, multivariate pseudo-$R^2_{(\text{GLMM})}$ for univariate traits revealed stronger selection in the inlet for S2 and across all intervals combined, stronger selection in the outlet in W2, and no difference in S1 and W1 (Fig. 5, Table S2). Based on geometric morphometrics (22 relative warps), the same analyses revealed that selection was strongest in the inlet in S2 ($CoV_{predicted}$ and

Table 1. Differences in general morphology between inlet and the outlet stickleback in our study.

Trait	Inlet	Outlet	$F_{df=1}$	Variance ratio (inlet/outlet)
Jaw length	0.26 ± 0.04	0.25 ± 0.03	109*	0.73*
Eye width	0.40 ± 0.02	0.38 ± 0.02	547.5*	0.69*
Pectoral fin width	0.25 ± 0.03	0.26 ± 0.03	12.3*	0.70*
Pectoral fin length	0.71 ± 0.05	0.70 ± 0.05	14.31*	0.62*
Pelvic spine length	0.65 ± 0.10	0.69 ± 0.11	103.7*	0.54*
Caudal fin length	0.64 ± 0.06	0.64 ± 0.07	0.57 ns	0.59*
Body depth	1.26 ± 0.06	1.15 ± 0.06	2100*	0.51*

Values depict the respective population mean and standard deviation of allometrically adjusted traits (in mm). *F*-values and their respective significance are based on ANOVA models. In addition, deviations of inlet/outlet variance ratios from 1 indicate higher trait variance in the outlet (*F*-test, $^*P < 0.001$).

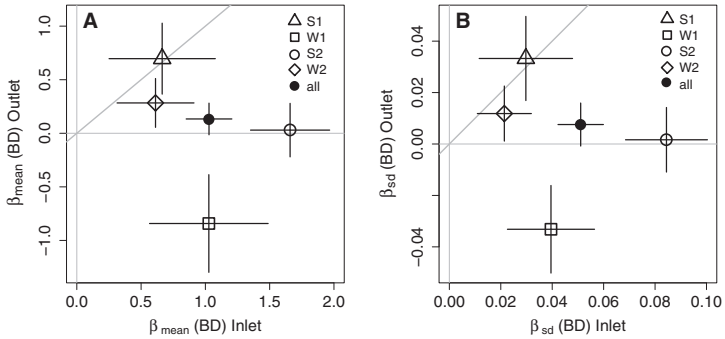

Figure 4. Strength of directional selection on body depth across all four intervals (S1-W2) and for all intervals combined (all). Points depict standardized selection coefficients (A: mean-standardized, B: variance-standardized) from binomial GLM(M)s and error bars depict standard deviations based on $n = 1000$ model simulation runs (see Methods).

Table 2. Differences in selection gradients between sites (inlet vs. outlet).

	Body depth		Site		Body depth × site	
Interval	LR χ^2	*P*	LR χ^2	*P*	LR χ^2	*P*
S1	6.89	8.6×10^{-3}	4.58	0.032	0.01	0.92
W1	1.06	0.301	0.15	0.69	7.88	0.005
S2	11.82	5.8×10^{-4}	13.64	2.2×10^{-4}	21.03	4.5×10^{-6}
W2	4.85	0.027	1.53	0.216	0.65	0.421
All	0.71	0.398	4.42	0.035	11.34	7.5×10^{-4}

Log likelihood ratio (LR) comparisons are based on binomial GLM estimates including body depth, site, and their interaction term as predictors.

pseudo-R^2, Fig. 5) and across all intervals, strongest in the outlet in W1, and not different in S1 and W2 (Fig. 5, Table S2). Finally, similar results to those just reported were evident in comparisons of Mahalanobis distances between fish that died versus survived—although larger confidence bounds meant that few significant differences were evident, except in S2 (Fig. 6).

Discussion

We were able to document significant viability selection acting in two wild populations of stream stickleback in the Misty system. Noteworthy selection was acting on the morphology, and this selection varied considerably through time. Although we do not know the cause of this temporal variation, its existence matches assertions that selection can be quite variable in nature (Siepielski et al. 2009, 2011, 2013), including in threespine stickleback (Reimchen and Nosil 2002, 2004). In addition, we found that selection varied across space (between the inlet and outlet stickleback), a phenomenon that also appears common in nature (Siepielski et al. 2013). We used this spatial variation to test the prediction outlined in the Introduction: Directional selection should be *stronger* in the outlet than in the inlet because

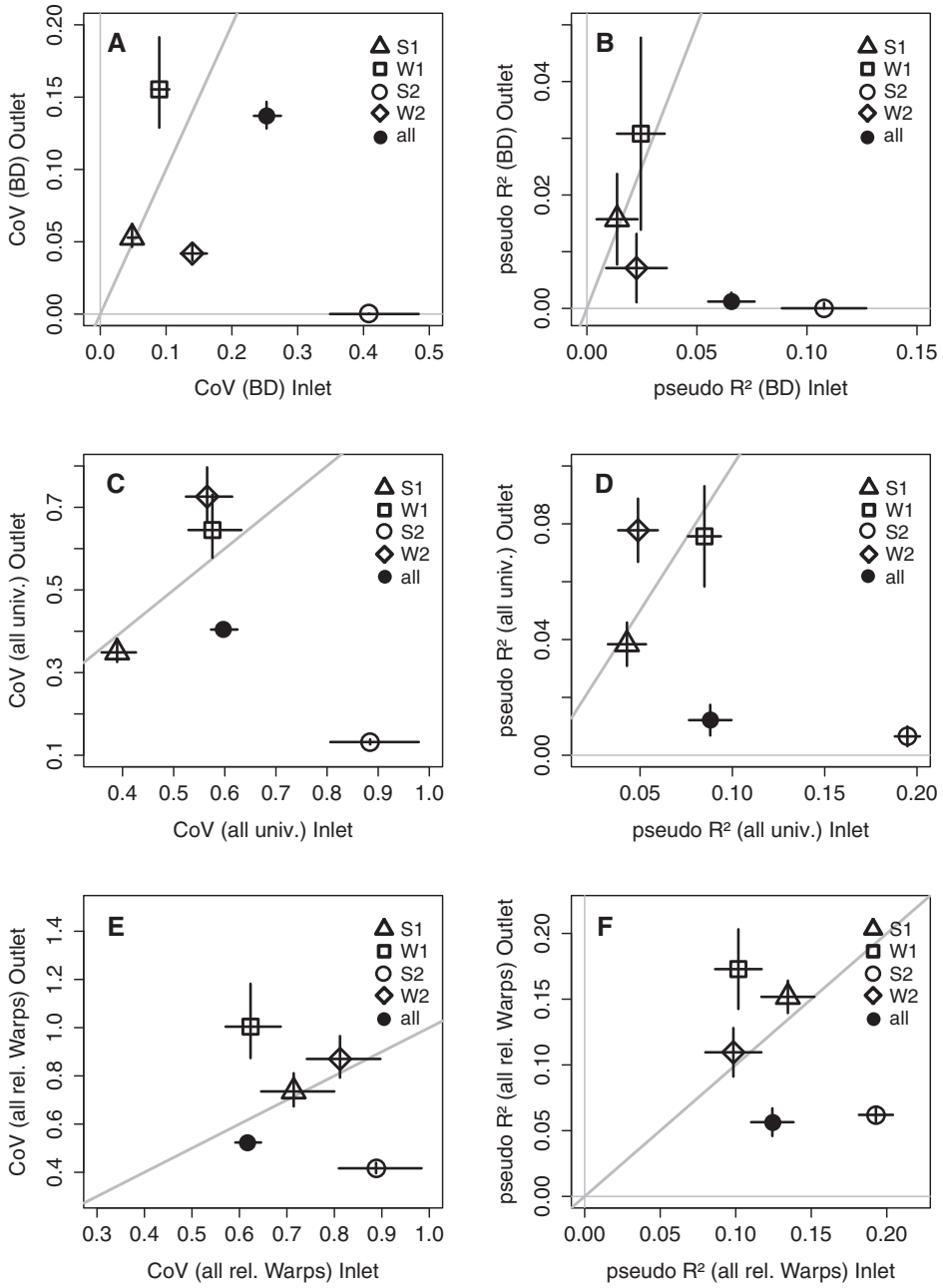

Figure 5. **Total strength of selection on body depth (panels A and B), all univariate traits (panels C and D), and all relative warps combined (panels E and F) measured as the amount of variance in fitness explained in binomial GLM(M)s. Measures are depicted across all for intervals (S1-W2) and for all intervals combined (all). Left side panels (A, C, and E): absolute amount of variation in fitness depicted as the coefficient of variation ($CoV_{predicted}$) in predicted fitness (error bars depict 95% CIs); right side panels (B, D, and F): relative amount of variation in fitness depicted as pseudo-R^2 and R^2_{GLMM} (error bars depict ½ standard deviations based on model bootstrapping).**

the former but not the latter experiences high maladaptive gene flow (Hendry et al. 2002; Moore et al. 2007). Our results contradicted this standard prediction, with directional selection being no stronger (and perhaps being weaker) in the outlet than in the inlet (Figs. 4–6, Table S2).

It would have been advantageous to have even larger sample sizes, higher recapture rates, more seasons, more sites, and more life-history stages (perhaps selection on juveniles is different). Moreover, our fitness estimate was based on apparent survival (fish that were not caught again were assigned a fitness of 0), which necessarily includes error variance from emigration and noncapture of live fish. Yet despite these caveats, our study was designed to minimize many of the constraints typically seen in attempts to estimate selection (see Introduction). Thus, our most

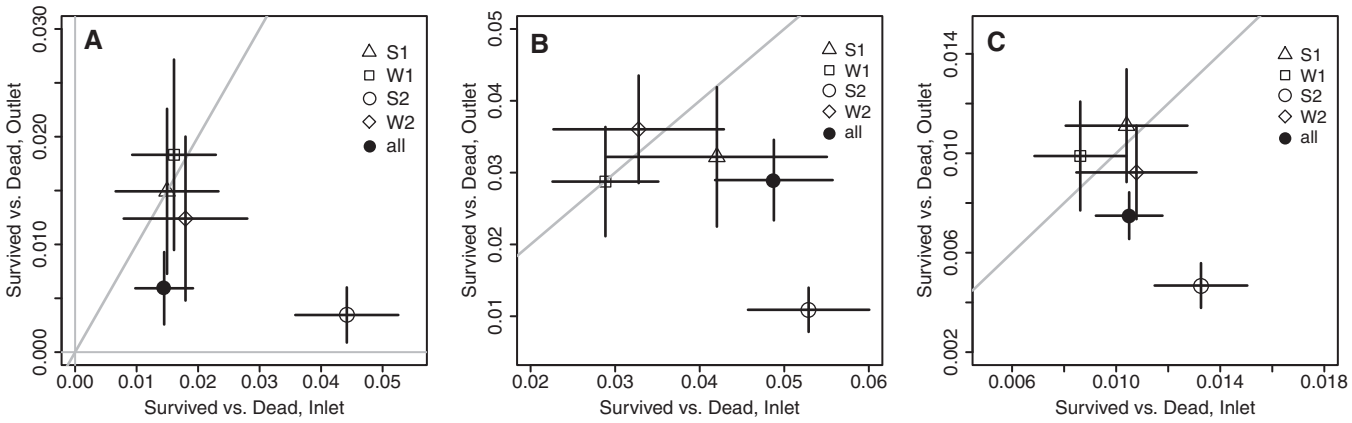

Figure 6. Strength of selection based on Mahalanobis distances between fish that survived and fish that died (not recaught) in the inlet versus the outlet for (A) body depth, (B) all univariate traits, and (C) all relative warps combined. Points depict distances at a specific site in a specific interval (S1-W2), and across all intervals (all). Error bars depict standard deviations based bootstrapping (see Methods).

basic conclusion (directional selection is *not* stronger in a population experiencing greater maladaptive gene flow) should be robust. This unexpected result suggests a revision of the standard prediction that increasing maladaptive gene flow should increase the strength of directional selection (Haldane 1930; Endler 1977; Lenormand 2002). We consider five possibilities.

First, migrants between populations might be phenotypically biased in the direction of the population to which they immigrate (Edelaar et al. 2008; Edelaar and Bolnick 2012). In our case, fish moving into the outlet stream might be relatively stream-like in their traits and thus might not cause strong maladaptation that would generate selection. Although phenotype-biased migration has been documented between lake and stream stickleback in another watershed (Bolnick et al. 2009), it unlikely explains our results. The main reason is that even the most stream-like Misty lake stickleback are far from typical stream morphology (Moore et al. 2007; Berner et al. 2008; Kaeuffer et al. 2012; see also Fig. 1) and so, even if migration was biased, gene flow would still be maladaptive. Moreover, a number of previous studies have shown quite clearly that Misty outlet stickleback are poorly adapted for their stream environment (Moore et al. 2007; Berner et al. 2008; Räsänen and Hendry 2014). We note, however, that the definitive test, a reciprocal transplant experiment between the inlet and the outlet, has not yet been conducted.

Second, the fitness function might be wider in the outlet than in the inlet, such that deviations from the optimal stream phenotype in the outlet have little fitness consequence. For instance, the upper outlet (0–500 m downstream of the Lake) has some lake-like features, such as slower and deeper water and some zooplankton, that are largely lacking in the inlet (Moore and Hendry 2005; Moore et al. 2007; Berner et al. 2008, 2009; Kaeuffer et al. 2012). As a result, selection against lake-like phenotypes might be relatively weak in this part of the outlet. Indeed, a reciprocal transplant experiment showed no fitness detriment for lake stickleback relative to outlet stickleback in *upper* outlet enclosures (Räsänen and Hendry 2014), a result consistent with weak selection. In this explanation, a wide, lake-like fitness function in the upper outlet is the reason for weak selection and, thus, high gene flow. However, the present study was conducted in the *lower* outlet (~1.2 km downstream of the lake), an area specifically chosen to resemble a stream-like environment comparable to the upper inlet (Fig. 1; Supplemental Material). Thus, the expectation for our two experimental sites is similar locations and widths of fitness peaks, arguing against this explanation. Nonetheless, it would be good for future work to formally construct adaptive landscapes (Arnold et al. 2001) for both environments.

Third, the morphologically maladapted (lake-like) phenotypes in the outlet potentially included not only immigrants from the lake but also the offspring of lake × outlet hybrid pairs. Given enough genetic differentiation between the lake and the outlet, these hybrids might be more heterozygous than residents and thus could benefit from heterosis (Richards 2000; Ebert et al. 2002). However, the magnitude of heterosis depends on the degree of inbreeding and the extent of genetic differentiation (Whiteley et al. 2015). Previous studies on the population genetic structure of Misty stickleback does not confirm these preconditions: no signs of inbreeding are evident in these populations and only minor genetic differentiation is evident between lake and outlet stickleback (Hendry et al. 2002; Moore et al. 2007; Roesti et al. 2012, 2014). Thus, although we cannot rule out this hypothesis, it does not seem particularly likely.

Fourth, antagonistic coevolution between stickleback and their enemies (e.g., parasites) could mean that lake stickleback immigrating to the outlet experience "enemy release" and therefore have higher fitness. Indeed, a number of studies of other systems have suggested that stickleback in lakes and streams

have different parasite communities (Kalbe et al. 2002; Eizaguirre et al. 2012) and that stickleback moving between environments are more heavily parasitized (MacColl and Chapman 2010; Stutz et al. 2014). Although we have not examined the fitness effects of parasites in our system, it is expected that parasite communities would be more divergent between the lake and inlet (upstream of the lake) than between the lake and outlet (downstream of the lake), again weakening the potential power of this explanation.

A final—and particularly intriguing—possibility is that high gene flow might *causally decrease* the strength of directional selection: the opposite effect to that normally posited (Lenormand 2002). This explanation starts with the realization that immigration can decrease resident population size (Boulding and Hay 2001; Tufto 2001), which should reduce competition and thus decrease the importance of adaptation. In this scenario, high gene flow flattens the adaptive landscape (without moving the location of the optimum) and thereby weakens selection. We are not aware of theoretical work specifically considering this possibility, and so we developed a population genetic model (see Appendix) of a well-adapted resident genotype on an island and a maladapted immigrant genotype from a continent. Conceptually, the model assumes adaptation to a sink habitat, specifically a black-hole sink, where there is no back-migration to source habitats (Gomulkiewicz et al. 1999; Holt et al. 2002). Migration therefore initially increases overall population size on the island and decreases the proportion of adapted resident genotypes. Moreover, competitive effects of genotypes on each other are asymmetric (e.g., Burgess et al. 2013), such that intergenotype competition is stronger than intragenotype competition for the immigrants because they are maladapted (see Appendix). These changes together depress mean fitness, which then decreases the strength of selection against immigrant genotypes. For instance, in the case of an asymmetric competition factor of $\alpha = 2$ (i.e., immigrant genotypes suffer twice as much from intergenotype competition vs. intragenotype competition), the equilibrium solution shows that increasing maladaptive gene flow (m) can decrease the strength of selection (β) against maladapted immigrant genotypes relative to adapted resident genotypes:

$$\beta = \frac{1}{2}(1 + \sqrt{1 - 4m}).$$

This model is an intentionally simplified caricature of the process intended to illustrate a previously unrecognized phenomenon. An empirical test of the model would be sensitive to many parameters that we did not record in this study, such as differences in immigration load and census size. For this reason, our study should not be considered a test of the model. Rather, the outcome of our study motivated the development of a theoretical model demonstrating that the standard prediction (gene flow increases selection) might not hold under some reasonable conditions (Fig. 7).

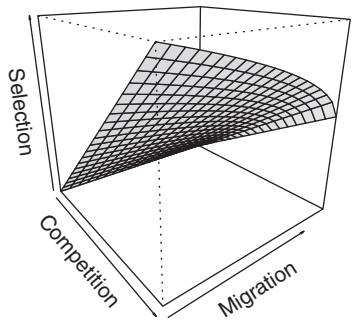

Figure 7. The effect of increasing migration on the strength of directional selection in a black-hole sink environment under the assumption of asymmetric competitive effects of resident and immigrant genotypes on each other. Note that, under these assumptions, the strength of selection decreases with increasing gene flow for a given asymmetric competition ratio between maladapted immigrants and well-adapted residents. The graph is based on the model outlined in the Appendix.

Having now considered, and generated some interesting explanations for, weak selection in the outlet population experiencing high maladaptive gene flow, we must also confront our finding that the presumptively well-adapted inlet population sometimes experiences strong selection. Indeed, the strongest episode of selection in our study was observed in the inlet and, when combining all episodes, selection was stronger in the inlet than the outlet (Figs. 4–6, Table S2). In explaining this result, we can first rule out the possibility that the inlet population is poorly adapted. Indeed, many studies have shown that Misty inlet stickleback are a typical stream-adapted population (Lavin and McPhail 1993; McPhail 1994; Hendry et al. 2002) that experiences very low gene flow from other populations (Hendry et al. 2002; Hendry and Taylor 2004; Moore et al. 2007; Kaeuffer et al. 2012; Hendry et al. 2013). As maladaptive gene flow is minimal, we are left with the other two explanations from the Introduction: constraints on adaptation or ongoing environmental change. Although it is hard to rule out constraints, the traits that are under selection certainly show substantial phenotypic variation (Fig. 3, Table 1) and are known to have a strong genetic basis in Misty lake stickleback (Sharpe et al. 2008; Berner et al. 2011; Hendry et al. 2011; see also Leinonen et al. 2011 for stickleback outside Vancouver Island). Environmental change therefore seems the more viable hypothesis.

Although we have not formally quantified environmental variation through time, small streams in this region are known to exhibit dramatic variation within and between years, especially with regard to water flow (e.g., Whitfield and Spence 2011). Coupled with what is likely a narrow fitness peak (given how strongly divergent the inlet population is from the lake population, Fig. 1),

environmental variation in the inlet could well impose selection that fluctuates through time and is sometimes quite strong. Importantly, such environmental variation would also partly account for the temporal fluctuations in selection we observed in the outlet (Fig. 3). As above, this argument highlights the importance of gaining additional information about the width of the fitness function, in addition to the position of the optimum.

SUMMARY AND CONCLUSIONS

Directional selection was not stronger in a population (Misty outlet) experiencing high maladaptive gene flow (from Misty lake) than in a population (Misty inlet) not experiencing maladaptive gene flow. Instead, directional selection was sometimes evident in the latter. These findings are in direct opposition to previous theoretical work, as well as a few empirical studies (Bolnick and Nosil 2007; Bolnick et al. 2008), arguing that high maladaptive gene flow should increase the strength of selection. This contradiction led us to consider explanations for why strong maladaptive gene flow might not increase selection. Of particular interest is the novel idea that high gene flow can causally reduce selection by broadening the fitness function—a result we demonstrated by means of a general population genetic model. An interesting implication of this effect is that very high gene flow can create situations that reinforce its negative effects on adaptation by reducing selection against maladaptive immigrants (and hybrids), which then further increases gene flow and further compromises local adaptation. A positive feedback is thereby generated that could lead to populations with very poor adaptation and very high gene flow despite divergent environments—as, indeed, seems to be the case for Misty outlet stickleback. We suggest that these effects are ripe for further theoretical, observational, and experimental studies.

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DATA ARCHIVING

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LITERATURE CITED

Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. Genetica 112–113:9–32.

Baker, J., K. Räsänen, J. S. Moore, and A. P. Hendry. 2013. Genetic and plastic contributions to trait divergence between parapatric habitats: female life-history traits in threespine stickleback within the Misty Lake system. Evol. Ecol. Res. 15:473–487.

Bell, G. 2008. Selection: the mechanism of evolution. 2nd ed. Oxford Univ. Press, Oxford, U.K.

Berner, D., D. C. Adams, A. C. Grandchamp, and A. P. Hendry. 2008. Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. J. Evol. Biol. 21:1653–1665.

Berner, D., A. C. Grandchamp, and A. P. Hendry. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution 63:1740–1753.

Berner, D., R. Kaeuffer, A. C. Grandchamp, J. A. M. Raeymaekers, K. Räsänen, and A. P. Hendry. 2011. Quantitative genetic inheritance of morphological divergence in a lake-stream stickleback ecotype pair: implications for reproductive isolation. J. Evol. Biol. 24:1975–1983.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. Ecology 86:1371–1384.

Bolnick, D. I., and P. Nosil. 2007. Natural selection in populations subject to a migration load. Evolution 61:2229–2243.

Bolnick, D. I., and O. L. Lau. 2008. Predictable patterns of disruptive selection in stickleback in postglacial lakes. Am. Nat. 172:1–11.

Bolnick, D. I., E. J. Caldera, and B. Matthews. 2008. Evidence for asymmetric migration load in a pair of ecologically divergent stickleback populations. Biol. J. Linn. Soc. 94:273–287.

Bolnick, D. I., L. K. Snowberg, C. Patenia, W. E. Stutz, T. Ingram, and O. L. Lau. 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. Evolution 63:2004–2016.

Both, C., S. Bouwhuis, C. M. Lessells, and M. E. Visser. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441:81–83.

Boulding, E. G., and T. Hay. 2001. Genetic and demographic parameters determining population persistence after a discrete change in the environment. Heredity 86:313–324.

Burt, A. 1995. The evolution of fitness. Evolution 49:1–8.

Burgess, S. C., R. S. Waples, and M. L. Baskett. 2013. Local adaptation when competition depends on phenotypic similarity. Evolution 67:3012–3022.

Carlson, S. M., and B. H. Letcher. 2003. Variation in brook and brown trout survival within and among seasons, species, and age classes. J. Fish Biol. 63:780–794.

Crespin, L., P. Berrebi, and J. D. Lebreton. 2002. Spatially varying natural selection in a fish hybrid zone. J. Fish Biol. 61:696–711.

Darwin, C. 1859. On the origin of species by means of natural selection. J. Murray, London.

Delcourt, M., K. Räsänen, and A. P. Hendry. 2008. Genetic and plastic components of divergent male intersexual behavior in Misty lake/stream stickleback. Behav. Ecol. 19:1217–1224.

Ebert, D., C. Haag, M. Kirkpatrick, M. Riek, J. W. Hottinger, and V. I. Pajunen. 2002. A selective advantage to immigrant genes in a *Daphnia* Metapopulation. Science 295:485–488.

Edelaar, P., and D. I. Bolnick. 2012. Non-random gene flow. Trends Ecol. Evol. 27:659–665.

Edelaar, P., A. M. Siepielski, and J. Clobert. 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution 62:2462–2472.

Eizaguirre, C., T. L. Lenz, M. Kalbe, and M. Milinski. 2012. Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. Ecol. Lett. 15:723–731.

Elliott, N. G., K. Haskard, and J. A. Koslow. 1995. Morphometric analysis of orange roughy (*Huplustetlius atlanticus*) off the continental slope of southern Australia. J. Fish Biol. 46:202–220.

Endler, J. 1977. Geographic variation, speciation and clines. Princeton Univ. Press, Princeton, N.J.

———. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, N.J.

Gelman, A., and J. Hill. 2006. Data analysis using regression and multilevel/hierarchical models. 1st ed. Cambridge Univ. Press, New York.

Gomulkiewicz, R., R. D. Holt, and M. Barfield. 1999. The effects of density dependence and immigration on local adaptation nad niche evolution in a black-hole sink environment. Theor. Pop. Biol. 55:283–296.

Gow, J. L., C. L. Peichel, and E. B. Taylor. 2007. Ecological selection against hybrids in natural populations of sympatric threespine sticklebacks. J. Evol. Biol. 20:2173–2180.

Hagen, D. W., and L. G. Gilbertson. 1973. Selective predation and the intensity of selection acting upon the lateral plates of threespine sticklebacks. Heredity 30:273–287.

Haldane, J. B. S. 1930. A mathematical theory of natural and artificial selection (Part VI, Isolation). Math. Proc. Cambridge Philos. Soc. 26:220–230.

Haller, B. C., and A. P. Hendry. 2014. Solving the paradox of stasis: squashed stabilizing selection and the limits of detection. Evolution 68:483–500.

Hansen, T. F. 2006. The evolution of genetic architecture. Annu. Rev. Ecol. Evol. Syst. 37:123–157.

Hansen, T. F., and D. Houle. 2008. Measuring and comparing evolvability and constraint in multivariate characters. J. Evol. Biol. 21:1201–1219.

Hendry, A. P., T. Day, and E. B. Taylor. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. Evolution 55:459–466.

Hendry, A. P., E. B. Taylor, and J. D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:1199–1216.

Hendry, A. P., and E. B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319–2331.

Hendry, A. P., K. Hudson, J. A. Walker, K. Räsänen, and L. J. Chapman. 2011. Genetic divergence in morphology-performance mapping between Misty Lake and Inlet stickleback. J. Evol. Biol. 24:23–35.

Hendry, A. P., R. Kaeuffer, E. Crispo, C. L. Peichel, and D. I. Bolnick. 2013. Evolutionary inferences from the analysis of exchangeability. Evolution 67:3429–3441.

Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? Evolution 58:2133–2143.

Hersch, E., and P. Phillips. 2004. Power and potential bias in field studies of natural selection. Evolution 58:479–485.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proc. Natl. Acad. Sci. U.S.A. 98:9157–9160.

Holt, R. D., R. Gomulkiewicz, and M. Barfield. 2002. The phenomenology of niche evolution via quantitative traits in a 'black-hole' sink. Proc. R. Soc. London B 270:215–224.

Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564–1571.

Kaeuffer, R., C. L. Peichel, D. I. Bolnick, and A. P. Hendry. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66:402–418.

Kalbe, M., K. M. Wegner, and T. B. H. Reusch. 2002. Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. J. Fish Biol. 60:1529–1542.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecol. Lett. 7:1225–1241.

Kingsolver, J. G., and S. E. Diamond. 2011. Phenotypic selection in natural populations: what limits directional selection? Am. Nat. 177:346–357.

Kingsolver, J. G., and H. E. Hoekstra. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Kitano, J., D. I. Bolnick, D. A. Beauchamp, M. M. Mazur, S. Mori, T. Nakano, and C. L. Peichel. 2008. Reverse evolution of armor plates in the threespine stickleback. Curr. Biol. 18:769–774.

Laake, J. L. 2013. Rmark: an R interface for analysis of capture-recapture data with MARK. AFSC Processed Rep 2013-01, 25p. Alaska Fish. Sci. Cent., NOAA, Seattle, WA.

Lavin, P. A., and J. D. McPhail. 1993. Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? Can. J. Zool. 71:11–17.

Leinonen, T., J. M. Cano, and J. Merilä. 2011. Genetics of body shape and armour variation in threespine sticklebacks. J. Evol. Biol. 24:206–218.

Lenormand, T. 2002. Gene flow and the limits to natural. Trends Ecol. Evol. 17:183–189.

Lin, J., T. P. Quinn, R. Hilborn, and L. Hauser. 2008. Fine-scale differentiation between sockeye salmon ecotypes and the effect of phenotype on straying. Heredity 101:341–350.

Lleonart, J., J. Salat, and G. J. Torres. 2000. Removing allometric effects of body size in morphological analysis. J. Theor. Biol. 205:85–93.

Lucek K., A. Sivasundar, D. Roy, and O. Seehausen. 2013. Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake–stream divergence in parapatric Swiss stickleback. J. Evol. Biol. 26:2691–2709.

Lynch, M. P., and A. F. Mensinger. 2013. Temporal patterns in growth and survival of the round goby (*Neogobius melanostomus*). J. Fish Biol. 82:111–124.

MacColl, A. D. C., and S. M. Chapman. 2010. Parasites can cause selection against migrants following dispersal between environments. Funct. Ecol. 24:847–856.

Marchinko, K. B., B. Matthews, M. E. Arnegard, S. M. Rogers, and D. Schluter. 2014. Maintenance of a genetic polymorphism with disruptive natural selection in stickleback. Curr. Biol. 24:1289–1292.

Matsumura, S., R. Arlinghaus, and U. Dieckmann. 2012. Standardizing selection strengths to study selection in the wild: A critical comparison and suggestions for the future. Bioscience 62:1039–1054.

McGee, M. D., D. Schluter, and P. C. Wainwright. 2013. Functional basis of ecological divergence in sympatric stickleback. BMC Evol. Biol. 13:277.

McPhail, J. D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. Pp. 399–437 *in* M. A. Bell and S. A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford Univ. Press, Oxford, U.K.

Moore, J. S., and A. P. Hendry. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. Evol. Ecol. Res. 7:871–886.

———. 2009. Can gene flow have negative demographic consequences? Mixed evidence from stream threespine stickleback. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364:1533–1542.

Moore, J. S., J. L. Gow, E. B. Taylor, and A. P. Hendry. 2007. Quantifying the constraining influence of gene flow on adaptive divergence in the lake-stream threespine stickleback system. Evolution 61:2015–2026.

Morrissey, M. B., and J. D. Hadfield. 2012. Directional selection in temporally replicated studies is remarkably consistent. Evolution 66:435–442.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for optaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4:133–142.

R Core Team. 2013. R: a language and environment for statistical computing. R Found. Stat. Comput., Vienna, Austria.

Raeymaekers, J. A. M., L. Delaire, and A. P. Hendry. 2009. Genetically based differences in nest characteristics between lake, inlet, and hybrid

threespine stickleback from the misty system, British Columbia, Canada. Evol. Ecol. Res. 11:905–919.

Raeymaekers, J.A.M., M. Boisjoly, L. Delaire, D. Berner, K. Räsänen, and A.P. Hendry. 2010. Testing for mating isolation between ecotypes: laboratory experiments with lake, stream, and hybrid stickleback. J. Evol. Biol. 23:2694–2798.

Räsänen, K., and A. P. Hendry. 2014. Asymmetric reproductive barriers and mosaic reproductive isolation: insights from Misty lake-stream stickleback. Ecol. Evol. 4:1166–1175.

Räsänen, K., M. Delcourt, L. J. Chapman, and A. P. Hendry. 2012. Divergent selection and then what not: the puzzle of missing reproductive isolation in Misty lake and stream stickleback. Int. J. Ecol. Vol. 2012, Article ID 902438, 14 pages doi:10.1155/2012/902438.

Ravinet, M., P. A. Prodöhl, and C. Harrod. 2013. Parallel and nonparallel ecological, morphological and genetic divergence in lake–stream stickleback from a single catchment. J. Evol. Biol. 26:186–204.

Reimchen, T. E., and P. Nosil. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. Evolution 56:2472–2483.

———. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58:1274–1281.

Reist, J. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. Can. J. Zool. 64:1363–1368.

Richards, C. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. Am. Nat. 155:383–394.

Roesti, M., A. P. Hendry, W. Salzburger, and D. Berner. 2012. Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. Mol. Ecol. 21:2852–2862.

Roesti, M., S. Gavrilets, A. P. Hendry, W. Salzburger, and D. Berner. 2014. The genomic signature of parallel adaptation from shared genetic variation. Mol. Ecol. 23:3944–3956.

Rohlf, F. J. 2005. tpsRelw, version 1.42. State Univ. New York, Stony Brook.

———. 2006. tpsDIG2, version 2.10. State Univ. New York, Stony Brook.

Schlaepfer, M. A., M. C. Runge, and P. W. Sherman. 2002. Ecological and evolutionary traps. Trends Ecol. Evol. 17:474–480.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.

———. 2000. The ecology of adaptive radiation. Oxford Series in Ecology and Evolution, Oxford Univ. Press, New York.

Schluter, D., T. Price, and L. Rowe. 1991. Conflicting selection pressures and life history trade-offs. Proc. R. Soc. Lond. B 246:11–17.

Sharpe, D., K. Räsänen, D. Berner, and A. P. Hendry. 2008. Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation. Evol. Ecol. Res. 10:849–866.

Siepielski, A. M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecol. Lett. 12:1261–1276.

Siepielski, A. M., J. D. DiBattista, J. A. Evans, and S. M. Carlson. 2011. Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. Proc. R. Soc. Lond. B 278:1572–1580.

Siepielski, A. M., K. M. Gotanda, M. B. Morrissey, S. E. Diamond, J. D. DiBattista, and S. M. Carlson. 2013. The spatial patterns of directional phenotypic selection. Ecol. Lett. 16:1382–1392.

Slatkin, M. 1985. Gene Flow in Natural Populations. Annu. Rev. Ecol. Syst. 16:393–430.

Stutz, W. E., O. L. Lau, and D. I. Bolnick. 2014. Contrasting patterns of phenotype-dependent parasitism within and among populations of threespine stickleback. Am. Nat. 183:810–825.

Thorpe, R. S., J. T. Reardon, and A. Malhotra. 2005. Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). Am. Nat. 165:495–504.

Tufto, J. 2001. Effects of releasing maladapted individuals: a demographic-evolutionary model. Am. Nat. 158:331–340.

Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback (*Gasterosteidae*) body shape. Biol. J. Linn. Soc. 61:3–50.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120–S139.

Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. Genetic rescue to the rescue. Trends Ecol. Evol. 30:42–49.

Whitfield, P. H., and C. Spence. 2011. Estimates of Canadian Pacific Coast runoff from observed streamflow data. J. Hydrol. 410:141–149.

Yoshimura, J., and V. A. A. Jansen. 1996. Evolution and population dynamics in stochastic environments. Res. Popul. Ecol. 38:165–182.

Zelditsch, M. 2012. Geometric morphometrics for biologists: a primer. 2nd ed. Elsevier Academic Press, London, UK.

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Appendix

For simplicity, we consider a haploid asexual species with two genotypes: A and a. The ideas presented here can be extended to diploid sexual populations but our purpose is simply to illustrate, in the simplest way possible, how one can get decreased selection arising from increased gene flow.

We use a continent-island model structure and employ the following differential equations to model the population sizes of the two genotypes on the island;

$$\begin{aligned} n_A{}' &= r_A n_A + m_A \\ n_a{}' &= r_a n_a + m_a \end{aligned}.$$

Here r_i is the per capita growth rate of genotype i, and m_i is the immigration rate of genotype i into the island from the continent. We take genotype A to be the type favored on the island.

The frequency of type A is $p = n_A/(n_A + n_a)$ and it is helpful to write the dynamics in terms of p and n, where n is the total population size $n = n_A + n_a$. We obtain

$$\begin{aligned} p' &= p(1-p)(r_A - r_a) + (1-p)\tfrac{m_A}{n} - p\tfrac{m_a}{n} \\ n' &= r_A pn + r_a(1-p)n + (m_A + m_a) \end{aligned}$$

where we can now identify $r_A - r_a$ as the selection gradient (i.e., the difference in fitness between the two genotypes).

Now, as a simple example, we assume that the per capita growth rates are $r_A = 1 - n_A - n_a$ and $r_a = 1 - 2n_A - n_a$. This embodies a linear form of density dependence such as that of classical Lotka–Volterra competition models, where the carrying capacity of each type on their own is normalized to be 1. The factor of 2 in the per capita growth rate of genotype a reflects an assumption that intergenotype competition is stronger than intragenotype competition for a individuals. The reason is that a individuals are poorly adapted for the island environment and so

are more negatively impacted by competition with a well-adapted A individual than another maladapted a individual. We realize that some models of competition make the opposite assumption (competition is stronger within than between types) but we are here specifically interested in competition between well-adapted and maladapted individuals for a particular resource, for which our assumption makes more sense.

Notice that intergenotype and intragenotype competition are of equal strength for A individuals. Thus, genotype a suffers disproportionately when genotype A is abundant. Put another way, for a fixed population size, the selection gradient for genotype A is an increasing function of the *frequency* of A as will be seen below. This can be seen by writing the per capita growth rates in terms of p and n:

$$\begin{aligned} r_A &= 1 - n \\ r_a &= 1 - n(1+p) \end{aligned}.$$

The selection gradient is then $\beta = r_A - r_a = np$.

For additional simplicity, we now make the assumption that all immigrants are maladapted ($m_A = 0$). Writing m_a simply as m, the complete model then becomes

$$\begin{aligned} p' &= p(1-p)np - p\tfrac{m}{n} \\ n' &= pn(1-n) + (1-p)n\,(1 - n(1+p)) + m \end{aligned}.$$

With this model one can show that the mutation-immigration balance at equilibrium is given by

$$\begin{aligned} n &= 1 \\ p &= \tfrac{1}{2}\left(1 + \sqrt{1 - 4\mathrm{m}}\right) \end{aligned}.$$

Interestingly, this result shows that the equilibrium population density is always 1 (the normalized carrying capacity) regardless of the immigration rate. When immigration occurs, the population size increases, but then the mean fitness drops such that, at equilibrium perfect compensation occurs and the population size returns to 1. The selection gradient at equilibrium is $\beta = r_A - r_a = np = \frac{1}{2}(1 + \sqrt{1 - 4m})$.

This result shows that the strength of selection decreases with increasing immigration rate. The reason is that, as the immigration rate increases, the equilibrium frequency of A decreases, and because the selection gradient is an increasing function of the frequency of A, this weakens the force of selection.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Map depicting sampling sites for the present mark-recapture study.
Figure S2. Upper panel.
Figure S3. Frequency distributions of individuals projected on the respective first discriminant function (DF1) for inlet versus outlet morphology for (A) all 22 relative warps, and (B) all univariate traits.
Figure S4. Comparison of the strength of directional selection on body depth in our study with selection measures from the Kingsolver et al. (2001) meta-analysis database.
Table S1. Goodness of fit for a candidate set of CJS mark-recapture models evaluated based on AICc scores according to whether survival probabilities (φ) and/or recapture probabilities (p) varied with time ($\sim$t), sampling site ($\sim$s), their interaction term ($\sim$t $\times$ s), or were constant through time ($\sim$1).
Table S2. Selection measures in both populations (inlet and outlet) across the four intervals (S1–W2) and for all intervals combined (All).