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Fitness differences between parapatric lake and stream stickleback revealed by a field transplant

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selection against migrants.

Abstract

Molecular comparisons of populations diverging into ecologically different environments often reveal strong differentiation in localized genomic regions, with the remainder of the genome being weakly differentiated. This pattern of heterogeneous genomic divergence, however, is rarely connected to direct measurements of fitness differences among populations. We here do so by performing a field enclosure experiment in threespine stickleback fish residing in a lake and in three replicate adjoining streams, and displaying weak yet heterogeneous genomic divergence between these habitats. Tracking survival over 29 weeks, we consistently find that lake genotypes transplanted into the streams suffer greatly reduced viability relative to local stream genotypes and that the performance of F1 hybrid genotypes is intermediate. This observed selection against migrants and hybrids combines to a total reduction in gene flow from the lake into streams of around 80%. Overall, our study identifies a strong reproductive barrier between parapatric stickleback populations, and cautions against inferring weak fitness differences between populations exhibiting weak overall genomic differentiation.

Introduction

Genomic studies exploring how molecular variation is influenced during adaptive divergence between populations residing in ecologically different habitats have become frequent (e.g. Nadeau *et al.*, 2012; Roesti *et al.*, 2012; Renaut *et al.*, 2013; Evans *et al.*, 2014; Soria-Carrasco *et al.*, 2014; Fraser *et al.*, 2015; Lamichhaney *et al.*, 2015). A common emerging pattern is heterogeneous genomic differentiation – that is, relatively strong population differentiation in localized regions of the genome and weak differentiation outside these regions. This pattern is typically interpreted as ecologically important loci experiencing divergent natural selection within a genomic background relatively homogenized by gene flow (Wu, 2001; Nosil *et al.*, 2009; Feder *et al.*, 2012). While such descriptions of genomic differentiation are valuable to shed light on the molecular complexity of adaptive divergence and to discover adaptation genes, they generally remain incomplete in that direct information about the fitness consequences of heterogeneous genomic divergence in nature is lacking (Barrett & Hoekstra, 2011). Establishing the link between genomic divergence and fitness differences among populations, however, is crucial to evaluate the promise of ecological genomics that mechanistic insights about adaptation and speciation can be derived from the examination of DNA sequence variation (Feder *et al.*, 2012).

In the present study, we address this link by experimentally quantifying fitness differences in nature among populations for which genomic divergence has been characterized. Specifically, we study populations of threespine stickleback fish (*Gasterosteus aculeatus* L.) residing in contiguous lake and stream habitats (i.e. in parapatry) within the Lake Constance basin in Central Europe (Berner *et al.*, 2010; Lucek *et al.*, 2010, 2012; Moser *et al.*, 2012). These populations have diversified ecologically: lake fish display a pelagic life style, exploiting zooplankton in the open water, whereas stream fish forage on benthic (substrate-dwelling) macro-invertebrates. This difference in foraging niches coincides with phenotypic divergence

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in foraging, predator defence and life history traits. However, some of the phenotypic divergence has been shown to be mainly plastic (Moser *et al.*, 2015), and traits generally exhibiting strong and consistent parallel lake–stream divergence in stickleback at a global scale (i.e. overall body shape, gill raker number; Reimchen *et al.*, 1985; Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013) have not evolved among lake and stream populations from the Lake Constance basin (Berner *et al.*, 2010; Lucek *et al.*, 2013). The relatively weak phenotypic divergence among our focal stickleback populations is mirrored by weak molecular differentiation: genome-wide high-density single nucleotide polymorphism (SNP) markers revealed baseline differentiation (i.e. genome-wide median F_{ST}) in multiple lake–stream population comparisons to range between 0.005 and 0.06 only (Roesti *et al.*, 2015). However, genomic differentiation appeared highly heterogeneous, with some genetic markers exhibiting strong differentiation.

A crucial question is now whether weak but heterogeneous genomic differentiation in stickleback from the Lake Constance basin is sufficient to cause substantial fitness trade-offs between the lake and stream habitats. This question is important because lake and stream stickleback populations in close contact have established as a strong system for studying the relationship between adaptive divergence and speciation (McKinnon & Rundle, 2002; Berner *et al.*, 2009; Hendry *et al.*, 2009). Nevertheless, adaptive divergence has so far been inferred only from the combination of phenotype–environment correlations (Reimchen *et al.*, 1985; Hendry & Taylor, 2004; Berner *et al.*, 2008, 2010; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013) and information on the genetic basis of trait divergence (Lavin & McPhail, 1993; Sharpe *et al.*, 2008; Berner *et al.*, 2011, 2014); unambiguous experimental demonstrations of whole-organism fitness differences between lake and stream stickleback are lacking (but see Hendry *et al.*, 2002 for suggestive results from Canadian populations, and Eizaguirre *et al.*, 2012 for adaptive divergence in immune genes). Furthermore, it remains uncertain how adaptive divergence contributes to the reproductive isolation (*RI*) driving and maintaining the (sometimes strong) marker-based genetic differentiation between lake and stream populations in close contact (Hendry *et al.*, 2009).

To inform these questions, we subject lake and stream stickleback from the Lake Constance basin to a transplant experiment to quantify fitness differences in nature. Combining the emerging results with recent genomic data, our study reveals the fitness correlate of heterogeneous genomic divergence and identifies powerful reproductive barriers between contiguous but ecologically different populations.

Materials and methods

Study design

The logic of our transplant study was to release juvenile stickleback from multiple stream populations into field enclosures in their stream of origin, together with lake fish and lake–stream F1 hybrids, and to track fitness until adulthood. We thus performed replicated 'local vs. foreign' experiments of local adaptation (Turesson, 1922; Clausen *et al.*, 1940; Kawecki & Ebert, 2004; Blanquart *et al.*, 2013). We expected that in the presence of adaptive divergence between the habitats, local stream fish should outperform foreign lake fish. Furthermore, assuming an overall additive genetic basis to potential fitness differences (which does not imply additivity at the underlying genetic loci; Lynch & Walsh, 1998), F1 hybrid performance should be intermediate between the pure populations. Although including the reciprocal experimental set-up – that is, transplanting stream fish into the lake – would have been desirable, the challenge of adequately reproducing pelagic foraging habitat in lake enclosures over many weeks imposed a unidirectional approach.

Our investigation considers four stickleback populations, including the one inhabiting Lake Constance (hereafter simply 'lake') and three from independent tributaries to the lake (NID, BOH and HOH; Fig. 1) (see also Berner *et al.*, 2010; Moser *et al.*, 2012, 2015; Roesti *et al.*, 2015). A single sample was adequate to represent the lake fish because this population is genetically well mixed (Moser *et al.*, 2012; Roesti *et al.*, 2015).

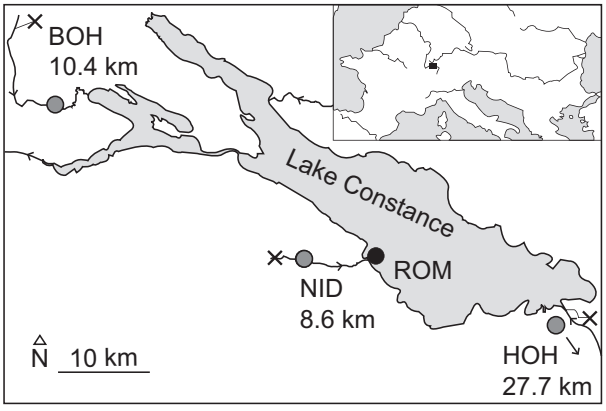

Fig. 1 Geographical situation of the study sites in the Lake Constance region (black rectangle in the insert map). The circles indicate the location of the three stream sites (NID, BOH and HOH) and the lake site (ROM) where stickleback were sampled to generate the experimental populations. The numbers indicate the approximate water distance from each stream site to the lake. The crosses indicate the location of the experimental stream enclosures.

Experimental fish

For our experiment, we used F2 individuals derived from the laboratory populations established by Moser *et al.* (2015). In brief, we first generated an F1 laboratory cohort in the spring of 2013 by artificially crossing field-caught reproductive individuals from each of the four study populations. We thus obtained 12 pure lake families and ten pure families from each of the three stream populations. The F1 individuals were pooled across families within each population, taking care to ensure an approximately similar contribution among families to each pool (details on the production of this F1 cohort and on husbandry are given in Moser *et al.*, 2015). After 1 year in the laboratory (i.e. between 26 April and 19 May 2014), individuals from the F1 cohort were sampled haphazardly to generate an F2 laboratory cohort. The latter again included the four pure populations, and additionally F1 hybrids between the lake and each stream population (i.e. seven cross-types in total). The number of replicate families was 16, 10, 16 and 5 for the lake, NID, BOH and HOH cross-types; and 10, 6 and 6 for lake–NID, lake–BOH and lake-HOH F1 hybrids. The juveniles of the F2 cohort were raised by pooling individuals from all replicate families of a given cross-type into a single aquarium. Rearing temperature was 16 °C, the light–dark photoperiod was 16 : 8, and the juveniles were fed live *Artemia* nauplii and frozen *Cyclops*, *Daphnia* and chironomid larvae ('bloodworms') *ad libitum*. Mortality in the laboratory was negligible.

Transplant experiment

Approximately 8–11 weeks post-hatch (24 July 2014), juveniles from the F2 cohort were transferred to field enclosures constructed in 2013 in each of the focal streams (Moser *et al.*, 2015). The enclosure sites were near the sites where the parental individuals of the laboratory lines had been sampled (Fig. 1), displayed habitat similar to the latter and harboured resident stickleback. Each stream site comprised three replicate enclosures. The enclosures were 6 m long and 1.5 m wide, and were built along the stream shore using perforated metal plates (4-mm-diameter holes, 58% passage), thus allowing the flow of water and small organisms across the enclosure walls (see Supporting Fig. S1 for enclosure photographs, and Moser *et al.*, 2015 for further details on the study sites and enclosure construction).

Prior to fish release, adult resident stickleback were removed from the enclosures by extensive minnow trapping (although small resident juveniles could enter and leave the enclosures). We then stocked each enclosure with a total of 90 juvenile stickleback from our laboratory lines, including 30 individuals from the focal stream, 30 lake individuals and 30 individuals from the corresponding F1 hybrid cross-type. Carefully monitoring body size in our F2 laboratory cohort ensured that all these experimental fish were too large to escape from the enclosures. To distinguish our experimental fish from residents, each cross-type was marked by clipping the first dorsal, the left pelvic or the right pelvic spine, using each clipping type once for every cross-type at each study site.

Starting five weeks post-release, the enclosures were visited on four occasions in intervals of eight weeks. The last visit occurred at the end of February 2015, after more than 200 days of experimental time, just before the onset of the breeding season (i.e. March; Moser *et al.*, 2015). During each visit, the enclosures were sampled in a standardized way by setting 14 minnow traps per enclosure 2 h before dusk and removing them 2 h after dawn on the following day. Recaptured experimental (i.e. marked) individuals were then assigned to cross-type, counted and weighed, providing survival and body mass as fitness measures. The number of nonexperimental fish captured in the enclosures (hereafter 'competitors') was also recorded. After this, all fish (experimental and competitors) were released back into their enclosures [except for the last visit when the experimental fish were killed with an overdose of Koi Med Sleep (phenoxyethanol; Fishmed, Rain, Switzerland) and preserved in Ethanol]. Across all study sites, this procedure was always completed within 3 days.

Supplementary measurements

To ensure the robustness of our experiment, three checks were performed. First, we haphazardly sampled and weighed 20 individuals from each cross-type the day before the release into the enclosures, which confirmed the absence of body mass differences among the cross-types at the onset of the experiment (Fig. S2). Second, after the standard sampling of the enclosures during the last field inspection, we continued sampling each enclosure with the same method. This extended sampling yielded few additional individuals (Fig. S3), indicating that our standard trapping scheme captured stickleback in the enclosures effectively. For consistency across the four sampling periods, these additional individuals were not considered for analysis (except in the calculation of *RI*, see below), although including them would only have strengthened our results (Fig. S3). Finally, we tested whether lake and stream stickleback differed in their intrinsic propensity to be captured by minnow traps. This test was conducted in mid-April 2015, after completing the field transplant experiment. We here stocked the three enclosures at the NID site with a similar number of marked, adult, field-caught lake and NID stream stickleback, each enclosure with a different total density (8, 16 and 24 individuals). After 12 h, we sampled the enclosures using the standard method described above, which recovered every single

released individual in each enclosure. This again confirmed the effectiveness of minnow trapping and showed that lake and stream fish were equally likely to be recaptured (this test was likely conservative, as the different growth environments of these field-caught lake and stream fish would be expected to exaggerate any genetically based behavioural difference). Together, these checks confirmed that our transplant experiment was very unlikely to be affected by methodological artefacts.

Furthermore, to characterize the natural abundance of resident stream stickleback at each study site, we applied our standard trapping scheme in the immediate area outside the enclosures in April 2014 and recorded the number of fish. Although this census was made outside our experimental period (i.e. July–February), the resulting counts should be roughly comparable to the number of competitors observed within the enclosures at the end of the experiment.

Data analysis

Our first prediction was that resident stream stickleback survive better in stream enclosures than foreign lake fish and that F1 hybrid survival falls between that of the pure lines. We therefore tested for differential survival among the experimental lines by fitting the number of survivors in the enclosures in a linear model with the terms cross-type, study site and their interaction. *P*-values for the model terms were obtained by permuting the number of survivors 9999 times and evaluating the observed F-statistics against their random distributions (Manly, 2007). For this test, we considered survival data from the fourth (last) sampling period only.

Our second prediction was that body mass attained during the experiment – an indirect fitness measure – was higher in stream than lake and hybrid fish. We thus tested for differences in body mass among the cross-types and study sites at the end of the experiment by permutation, using the same model structure as for survival. This test, however, considered only stream fish and hybrids because only a single lake survivor was recovered at the NID and BOH sites.

Differences among the study sites and sampling periods in the number of competitors present in the enclosures were tested analogously based on a repeated measures model with study site as factor and sampling time as within-enclosure effect.

To examine to what extent performance differences among the experimental lines reduced gene flow from the lake into the streams, we calculated the strength of unidirectional *RI* using the formula 4A from Sobel & Chen (2014):

$$RI = 1 - 2 \times (H/(H + C))$$

We here substituted the number of lake and stream survivors at the end of the experiment for *H* (the common shorthand notation for 'heterospecific') and *C* ('conspecific') to quantify the reproductive barrier due to viability selection against lake immigrants. Analogously, substituting the number of hybrid survivors for *H* expressed *RI* due to selection against hybrids. Note that *RI* varies linearly from 1 (complete *RI*, here corresponding to an absolute barrier to gene flow from the lake into the streams) to −1 (maximum possible gene flow from the lake into the streams). We calculated this metric both globally, combining survival data from all enclosures and streams, and separately for each stream, combining data from all enclosures. Graphing and analyses were performed in R (R Development Core Team, 2014). All raw data are available from the Dryad Digital Repository (doi:10.5061/dryad.86fj0).

Results

As predicted for local adaptation, survival in the stream enclosures was consistently higher in local stream stickleback than in foreign lake fish, and F1 hybrid survival was intermediate (Fig. 2, upper row) (cross-type effect, permutation $P = 0.0151$). Most strikingly, at the NID site, experimental lake fish were already essentially eliminated 13 weeks after the release, whereas stream and hybrid fish were still present in all enclosures. Survival also differed among the streams ($P = 0.0006$; cross-site interaction: $P = 0.134$), with the highest survival occurring at the HOH site. At all sites, mortality was most severe during the first sampling interval.

On average, body mass of experimental stickleback increased more than three-fold in the course of the experiment. However, contrary to our expectation, we found no difference in body mass between the pure stream and the F1 hybrid crosses, nor among the study sites (all model terms $P > 0.162$) (Fig. 2, middle row).

The number of competitors in the enclosures differed clearly among the stream sites ($P = 0.0006$), declining from NID (mean over enclosures and time points: 81) to BOH (61) and HOH (10) (Fig. 2, bottom row). We also observed a decrease over time ($P = 0.0031$), driven primarily by the NID and BOH sites (site–time interaction: $P = 0.0016$). We suspect that this temporal decline is underestimated by our methodology, as a proportion of small juvenile fish in the beginning of the experiment escaped our trapping. The number of competitors within the enclosures further mirrored the natural abundance of stickleback present at the study sites, as revealed by sampling outside the enclosures (Fig. 2, bottom row, week 29).

The survival differences among the cross-types by the end of the experiment implied a substantial barrier to gene flow: in the global analysis, *RI* attributable to selection against lake immigrants and against lake–stream hybrids was 0.67 and 0.4. The corresponding

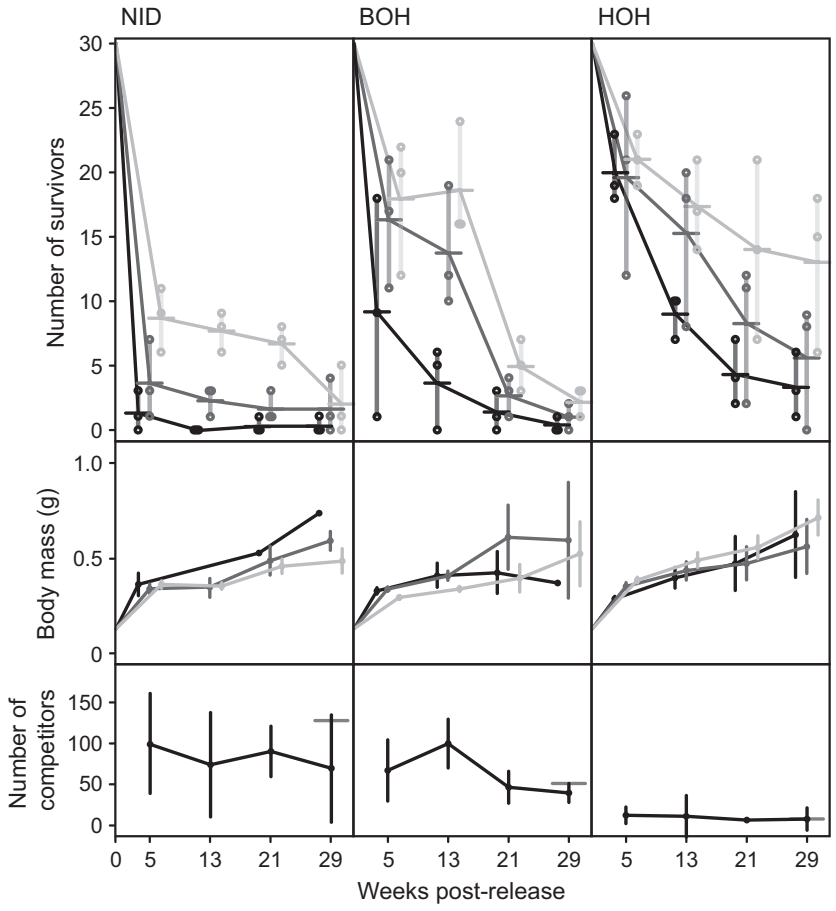

Fig. 2 Upper row: number of lake (black), stream (light grey) and F1 hybrid (dark grey) stickleback surviving in the field enclosures during the transplant experiment at each study site. The circles represent the raw survivor counts in each replicate enclosure, the vertical bars connect the minima and maxima, and the horizontal bars are the means across the enclosures (connected by lines between the sampling periods; note that because of incomplete sampling, mean survivor number sometimes increases slightly from one sampling period to the next). Middle row: change in body mass along the experiment. Dots are means across the replicate enclosures (using within-enclosure averages as data points), and error bars are the associated parametric 95% confidence intervals (note that after week 5, lake stickleback at the NID site are represented by a single individual only). Bottom row: number of competitors (i.e. nonexperimental stream-resident stickleback) captured within the enclosures during the experiment. Dots and error bars are means and 95% confidence intervals across the replicate enclosures. The grey horizontal bars at week 29 indicate the number of stickleback captured outside the enclosures (this latter census was made slightly outside our experimental period, see Materials and Methods).

site-specific values were 0.71 and 0.1 for NID, 0.82 and 0.54 for BOH, and 0.63 and 0.42 for HOH.

Discussion

Local adaptation in lake–stream stickleback

Our objective was to test for fitness differences between lake and stream stickleback and their hybrids through a replicated transplant experiment in nature. The emerging pattern is clear and consistent: local stream fish display higher survival than lake fish, and F1 hybrids are intermediate between these populations. Although our experimental individuals were confined in field enclosures, this is unlikely to compromise the generality of our findings: the enclosures were relatively large and permeable to prey organisms, and importantly, stickleback densities within the enclosures were comparable to those outside the enclosures. The experiment can be expected to have reproduced the selective conditions in the streams adequately.

We thus provide the first demonstration of adaptive divergence between lake and stream stickleback at the level of whole-organism performance and contribute to the scant body of direct experimental evidence of fitness differences among natural populations of vertebrates (e.g. Schluter, 1995; Gomez-Mestre & Tejedo, 2003). Our finding of strong fitness differences between

lake and stream stickleback within stream habitats differs from merely suggestive differences found in a transplant study using Canadian lake–stream populations (Hendry *et al.*, 2002). The different outcomes, however, are likely attributable to different methodologies: the latter study used adult, field-caught experimental fish and ran for a much shorter time, and therefore probably lacked power to detect selection.

Having conducted our experiments in streams only, we recognize that for a formal demonstration of local adaptation (as opposed to stream fish being *unconditionally* fitter than lake fish), a reciprocal transplant of lake and stream populations across both habitats would have been needed (Kawecki & Ebert, 2004; Blanquart *et al.*, 2013). However, molecular analyses have established that the Lake Constance population is evolutionarily derived from a stream ancestor and has experienced genomically wide-spread selective sweeps in its novel habitat (Roesti *et al.*, 2015). This makes clear that the lake habitat is challenging for stream fish. Moreover, Lake Constance fish have diverged from the tributary populations in ecologically important traits such as defensive lateral plating and gill raker length (Berner *et al.*, 2010; Moser *et al.*, 2012; Lucek *et al.*, 2013), differentiation generally known to have a strong genetic basis in stickleback (Colosimo *et al.*, 2005; Berner *et al.*, 2014; Glazer *et al.*, 2015; Roesti *et al.*, 2015). The reciprocal expectation of higher fitness of lake than stream or hybrid fish in the lake habitat thus appears highly plausible.

Contrary to our prediction, body mass, our indirect fitness measure, did not differ among the experimental populations in the end of the experiment. An obvious explanation is that individuals achieving poor growth were eliminated continuously, a possibility we cannot evaluate because our population-level marking did not allow tracking survival and growth of individual fish.

Our experiment further suggests an interesting detail about the nature of selection in lake–stream stickleback. Specifically, selection against lake fish (and hybrids) appeared relatively relaxed at the study site HOH that also displayed the lowest density of resident stickleback, as observed both within and outside the enclosures. This is most evident when considering the proportion of surviving lake fish relative to the total number of individuals (experimental and competitors) recovered at the end of the experiment, pooled across all replicate enclosures. This proportion was vanishingly low (0.005 and 0.008) in the NID and BOH systems displaying relatively high competitor densities, but substantial (0.12) at HOH where resident stickleback were much less abundant. (We suspect that the low natural abundance of stream residents at HOH is due to a shortage of breeding habitat; this stream site exhibits higher water flow and less organic litter and vegetation than the two other sites; Dario Moser and Daniel Berner, personal observation). Similarly, the overlap in survival among the cross-types early in the experiment was highest at the HOH site. This suggests that dispersing lake fish perform well in relatively sparsely populated streams, but are eliminated rapidly from streams in which a dense locally adapted population is present. We thus hypothesize that selection is density-dependent and driven by intraspecific resource competition – a factor generally considered important to ecologically based *RI* (Schluter, 2000; Nosil, 2012). Although density-dependent selection in this stickleback system needs to be confirmed more directly, our study highlights the value of replicating selection studies across multiple habitats (Wade & Kalisz, 1990).

Genomic differentiation and reproductive isolation

Our laboratory stickleback lines producing the experimental fish had spent more than an entire life cycle under standardized conditions. The fitness differences observed in the field enclosures must therefore be attributed largely to genetic differentiation between the lake and stream populations. Fortunately, genome-wide information about this differentiation is available: the lake and two of the three stream populations (NID and BOH) have been genotyped previously at high-density SNP markers for demographic analysis, and to study signatures of selection in the genome (Roesti *et al.*, 2015). This provides the opportunity to relate fitness differences to genomic divergence in the same populations.

To this end, we here re-use the SNP data to characterize the genome-wide distribution of genetic differentiation (F_{ST}) for both the lake–NID and the lake–BOH population comparisons. These distributions (Fig. 3) highlight the weak overall lake–stream differentiation in both population comparisons (genome-wide median F_{ST}, lake–NID: 0.013; lake–BOH: 0.005), the absence of complete allele frequency shifts (i.e. $F_{ST} = 1$), but that a small proportion of loci nevertheless exhibit strong lake–stream differentiation (up to 0.84 and 0.67 in the two comparisons). (A very similar F_{ST} distribution can be expected for the lake-HOH comparison, as microsatellite-based differentiation in this population pairing is intermediate between the lake–NID and lake–BOH comparisons; Moser *et al.*, 2012.) We thus find strong fitness differences in population pairs in which overall genetic differentiation would generally be considered very weak. For example, using fully comparable methodology, genome-wide baseline F_{ST} was estimated as high as 0.15 between neighbouring lake and stream stickleback populations from Vancouver Island, Canada (Roesti *et al.*, 2012).

The weak and heterogeneous genomic differentiation in our lake–stream systems directly translates to powerful pre- and post-zygotic reproductive barriers:

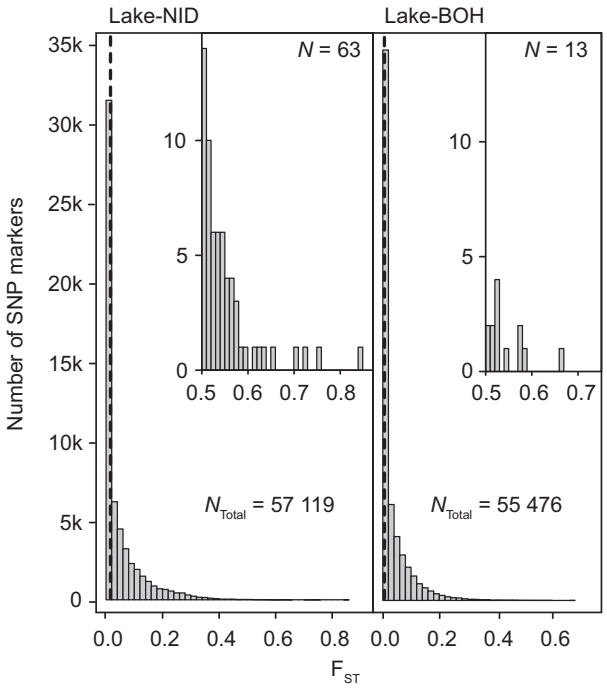

Fig. 3 Distribution of the genetic differentiation (F_{ST}) at single nucleotide polymorphisms (SNPs) in lake–stream comparisons involving the lake (sample size: 25 individuals) and two of our focal stream populations (NID and BOH, 24 and 22 individuals). Separate histograms are shown including all SNPs, and including only those SNPs exhibiting $F_{ST} \geq 0.5$ (inserts), along with the corresponding marker numbers. The dotted lines represent genome-wide median F_{ST} (lake–NID: 0.013; lake–BOH: 0.005). Data re-analysed from Roesti *et al.* (2015).

averaged across the streams, selection against migrants (Coyne & Orr, 2004; Hendry, 2004; Nosil *et al.*, 2005) reduces gene flow from the lake into the stream populations by approximately 70% relative to the absence of fitness differences between the populations. Furthermore, the ecological inferiority of F1 hybrids resulting from mating between dispersers from the lake and stream residents reduces gene flow by another 40%. Combining these two reproductive barriers sequentially (Coyne & Orr, 1989; Sobel & Chen, 2014), adaptive divergence drives total *RI* in the order of 0.8. This strong ecological barrier to gene flow offers an answer to the long-standing question of how lake and stream stickleback can maintain (often striking) genetic and phenotypic differentiation in close contact (Reimchen *et al.*, 1985; Berner *et al.*, 2009; Bolnick *et al.*, 2009; Eizaguirre *et al.*, 2009; Hendry *et al.*, 2009; Hanson *et al.*, 2016).

We emphasize, however, that it remains unclear to what extent the components of *RI* identified in our study actually operate in nature, as they require that lake stickleback disperse into tributaries (selection against migrants) and interbreed with stream fish (selection against hybrids). These assumptions appear plausible because the Lake Constance population invades tributaries during the reproductive period. Indeed, anecdotal evidence indicates overlap in breeding habitat between lake and stream stickleback at least in the BOH stream. Nevertheless, our present insights should be complemented with experimental information on how the opportunity for gene flow is modified by dispersal behaviour (Edelaar & Bolnick, 2012; Webster *et al.*, 2012; Berner & Thibert-Plante, 2015; see Bolnick *et al.*, 2009 for a habitat preference study in Canadian lake–stream stickleback) and by sexual interactions (Eizaguirre *et al.*, 2009; Raeymaekers *et al.*, 2010; Moser *et al.*, 2015).

Conclusions

We demonstrate strong genetically based fitness differences between neighbouring lake and stream stickleback populations despite weak – but heterogeneous – genomic differentiation. Our study thus highlights the challenge of predicting the magnitude of adaptive divergence based on genetic markers only. We further show that adaptive divergence translates to strong ecologically based reproductive barriers. Future studies are needed to compare the relative importance of these and other reproductive barriers in stickleback from the Lake Constance basin, and to experimentally measure fitness differences in other lake–stream systems, including those known to exhibit stronger phenotypic and genomic differentiation.

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Supporting information

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

Figure S1 Photographs of enclosures used for the field transplant experiment.

Figure S2 Body mass and standard length of subsamples from all seven experimental cross types 1 day before release.

Figure S3 Number of survivors, body mass, and number of competitors at the end of the transplant experiment, based on standard vs. extended sampling within the enclosures.

Data deposited at Dryad: doi: 10.5061/dryad.86fj0

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