Assessing reproductive isolation using a contact zone between parapatric lake-stream stickleback ecotypes

D. HANSON*, J.-S. MOORE†, E. B. TAYLOR‡, R. D. H. BARRETT* & A. P. HENDRY*

*Redpath Museum and Department of Biology, McGill University, Montreal, QC, Canada
†Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, QC, Canada
‡Department of Zoology and Beaty Biodiversity Museum, University of British Columbia, Vancouver, BC, Canada

Abstract

Ecological speciation occurs when populations evolve reproductive isolation as a result of divergent natural selection. This isolation can be influenced by many potential reproductive barriers, including selection against hybrids, selection against migrants and assortative mating. How and when these barriers act and interact in nature is understood for relatively few empirical systems. We used a mark–recapture experiment in a contact zone between lake and stream three-spined sticklebacks (*Gasterosteus aculeatus*, Linnaeus) to evaluate the occurrence of hybrids (allowing inferences about mating isolation), the interannual survival of hybrids (allowing inferences about selection against hybrids) and the shift in lake-like vs. stream-like characteristics (allowing inferences about selection against migrants). Genetic and morphological data suggest the occurrence of hybrids and no selection against hybrids in general, a result contradictory to a number of other studies of sticklebacks. However, we did find selection against more lake-like individuals, suggesting a barrier to gene flow from the lake into the stream. Combined with previous work on this system, our results suggest that multiple (most weakly and often asymmetric) barriers must be combining to yield substantial restrictions on gene flow. This work provides evidence of a reproductive barrier in lake–stream sticklebacks and highlights the value of assessing multiple reproductive barriers in natural contexts.

Introduction

Ecological speciation is increasingly recognized as an important mechanism generating Earth's biodiversity (Funk *et al.*, 2006; Nosil, 2012; Shafer & Wolf, 2013; Faria *et al.*, 2014). In this process, reproductive isolation evolves between populations as a result of ecologically based divergent selection (Schluter, 2000; Rundle & Nosil, 2005; Nosil, 2012). However, some authors have expressed reservations as to the ubiquity and power of ecological speciation (e.g. Coyne & Orr, 2004; Hendry, 2009, 2016; Rundell & Price, 2009; Svensson, 2012). One source of this uncertainty is that a number of

Correspondence: Dieta Hanson, Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke St. W., Montreal, QC H3A 0C4, Canada.
Tel.: +1 514 398 0856; fax: +1 514 398 3185;
e-mail: dieta.hanson@mail.mcgill.ca

systems putatively undergoing ecological speciation have yet to be characterized in terms of their ecologically based reproductive barriers. Identifying these barriers is a key to clarifying the prevalence of ecological speciation. The potential barriers are many, including natural and sexual selection against migrants, habitat choice, differences in reproductive timing (allochrony), and natural and sexual selection against hybrids (Coyne & Orr, 2004; Nosil, 2012). These and other potential barriers differ in many aspects: when they act during reproduction (early-acting barriers can be more important), the ease with which they can evolve (depending on recombination rates, population size and levels of genetic variation, among others), the extent to which they ultimately generate total and irreversible isolation (the most likely being intrinsic genetic incompatibilities) and in many other properties critical to speciation (Coyne & Orr, 2004; Nosil, 2012). As a result, identifying barriers to reproduction in natural systems is an

important area of research needed to advance our understanding of ecological speciation (Schluter, 2001; Nosil, 2012; Faria *et al.*, 2014).

Many of the potential reproductive barriers during ecological speciation can only be assessed in nature when the diverging groups (populations, incipient species or established species) come into contact with each other or the divergent environment, with the most obvious such barriers being natural and sexual selection against migrants and hybrids. Thus, studies of reproductive barriers between populations that do not naturally encounter such situations often bring the different groups into the laboratory (e.g. Honma & Tamura, 1984; Nosil *et al.*, 2002; Boughman *et al.*, 2005; Langerhans *et al.*, 2007; Lowry *et al.*, 2008; Castillo *et al.*, 2015). Although such studies have revealed many strong reproductive barriers (Schluter, 2000; Nosil, 2012), they are always open to the criticism that barriers in the laboratory might bear little resemblance to barriers in nature. An improvement is to create situations of artificial contact with divergent populations or environments in more natural settings, such as enclosures in the wild or mesocosms (e.g. Schluter, 2000; Via *et al.*, 2000; Hendry *et al.*, 2002; Thorpe *et al.*, 2005; Schwartz *et al.*, 2010; Moser *et al.*, 2016). Although such experiments are undoubtedly closer to the natural situation, they remain attended by questions of realism, such as how many and when individuals are moved and the artificiality of experimental arenas – even in nature. Thus, investigators have long sought, and often profitably employed, regions of natural (unmanipulated) overlap between populations and environments.

Contact zones, where groups undergoing putative ecological speciation naturally encounter one another and have the opportunity to interbreed, provide a situation in which reproductive barriers can be effectively studied in nature. By working with contact zone populations, investigators can assess various reproductive barriers, such as selection against migrants, the natural production of hybrids and selection acting against hybrids. For instance, contact zones have been used to document natural hybridization followed by selection against hybrids in a diverse array of taxa, including plants (Tastard *et al.*, 2012; Hopkins *et al.*, 2014; Lindtke *et al.*, 2014), invertebrates (Rolán-Alvarez *et al.*, 1997; Toro *et al.*, 2004), amphibians (Alexandrino *et al.*, 2005) and mammals (Shurtliff *et al.*, 2014). Contact zones in which hybrids occur are particularly useful because they include populations that have not completed speciation, and are instead intermediate on the continuum from panmictic to completely isolated species (Hendry *et al.*, 2009). Furthermore, as opposed to those created artificially, natural hybrids will not be subject to genotype-by-environment interactions during development in a laboratory that may produce phenotypes unlike those encountered in nature and thus are more valuable for understanding natural processes. In

this study, we sought to employ the power of a natural contact zone between genetically divergent three-spined sticklebacks (*Gasterosteus aculeatus*, Linnaeus) populations to test for some of the reproductive barriers that might be contributing to restricted gene flow in parapatry.

Three-spined sticklebacks are commonly used for studying ecological speciation owing to their repeated colonization of different environments, followed by adaptive divergence into 'ecotypes' that show varying degrees of reproductive isolation (McKinnon & Rundle, 2002; Hendry *et al.*, 2009). Our specific focus is on lake and stream ecotype pairs, where genetic markers have revealed restricted gene flow in parapatry in many independent watersheds (Hendry *et al.*, 2002; Hendry & Taylor, 2004; Berner *et al.*, 2009; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013; Roesti *et al.*, 2015). Despite these, sometimes large genetic differences on even very small spatial scales, ecologically based reproductive barriers have proven hard to identify. Mate choice (Raeymaekers *et al.*, 2010; Räsänen *et al.*, 2012) and allochronic isolation (Hanson *et al.*, 2016) are two barriers that seem to be entirely absent. Selection against migrants appears to be asymmetric and at least sometimes very weak (Hendry *et al.*, 2002; Räsänen & Hendry, 2014; Moser *et al.*, 2016). Habitat selection is important in at least one system (Bolnick *et al.*, 2009; Jiang *et al.*, 2015), but its generality is unknown. Faced with this lack of clear consensus, we here consider natural selection against hybrids, one of the most important ecologically based reproductive barriers in many taxa (Coyne & Orr, 2004; Nosil, 2012).

Several *a priori* reasons exist to suspect selection against hybrids, as work on other stickleback systems has shown. For example, in freshwater–anadromous pairs, Jones *et al.* (2006) found very low gene flow despite the presence of hybrids and interpreted this pattern to mean that post-zygotic barriers such as selection against hybrids must be at least as important as prezygotic barriers. Similarly, many studies have shown that benthic–limnetic stickleback hybrids typically show inferior growth rates to the locally adapted pure type in each parental habitat (review: Hendry *et al.*, 2009; but see Taylor *et al.*, 2012). Correspondingly, the frequency of benthic–limnetic hybrids in lakes has been shown to decrease through the life cycle, suggesting that hybrids suffer a survival disadvantage (Gow *et al.*, 2007). Finally, environmental change that reduced selection against hybrids contributed to the collapse of the Enos Lake benthic–limnetic pair (Behm *et al.*, 2010). This evidence from other stickleback systems makes it reasonable to suppose that lake–stream hybrids might also suffer a fitness disadvantage.

Several previous studies have considered lake–stream hybrids but were largely restricted to laboratory-raised fish. First, intrinsic genetic incompatibities that would prevent the production of hybrids do not appear

significant in some populations (Lavin & McPhail, 1993; Hendry *et al.*, 2002). Second, although hybrids show intermediate mating behaviours in the laboratory, they do not appear to suffer a mating disadvantage (Raeymaekers *et al.*, 2010). Third, artificial crosses have found that hybrids are generally intermediate between the lake and stream ecotypes with respect to genetically based morphological traits (Hendry *et al.*, 2002; Berner *et al.*, 2011), which suggests that they would suffer ecologically based survival deficits in nature. Moving closer to nature, laboratory-reared F1 hybrids released into stream field enclosures had survival rates intermediate to pure stream and lake ecotypes (Moser *et al.*, 2016), although similar experiments in the lake would be needed to test for the ecological dependency of this effect. Finally, classic work found that morphological lake–stream hybrids are rare in nature (Reimchen *et al.*, 1985; Lavin & McPhail, 1993). In short, previous work on lake–stream sticklebacks has left open the question of the importance of selection against hybrids as an ecologically based reproductive barrier, especially as no studies have examined the fate of hybrids in nature.

We studied lake and inlet stream sticklebacks in the Misty system on northern Vancouver Island, British Columbia, Canada. Despite their very close physical proximity (parapatry), these two populations exhibit many genetically based phenotypic differences that indicate strong adaptive divergence (Lavin & McPhail, 1993; Hendry *et al.*, 2002, 2011; Sharpe *et al.*, 2008; Berner *et al.*, 2009; Kaeuffer *et al.*, 2012; Oke *et al.*, 2016). They also exhibit high neutral genetic divergence indicative of strong reproductive isolation (Thompson *et al.*, 1997; Hendry *et al.*, 2002; Moore *et al.*, 2007; Kaeuffer *et al.*, 2012). Yet the specific reproductive barriers driving this distinction have been, as summarized above, hard to establish. The lake and inlet stream environments in the Misty system are highly distinct (Moore *et al.*, 2007; Kaeuffer *et al.*, 2012), but meet where the stream divides into narrow, deep channels that cut a grass-dominated marsh before emptying into the lake. Within this environment, we use morphology and genetics to first test whether hybrids are indeed present – indicating natural hybridization. We then use a mark–recapture experiment to estimate survival and ask whether selection acts against these hybrids. Finally, we attempt inferences about the specific nature of selection using temporal shifts in the lake-like vs. stream-like genetic and morphometric properties of contact zone fish.

Materials and methods

In 2009, we set minnow traps in the channels that lead from the inlet stream (hereafter 'stream') to the lake to determine whether a contact zone existed. We found many sticklebacks in a location approximately 40 m into the marsh from the edge of the lake (Fig. 1). On
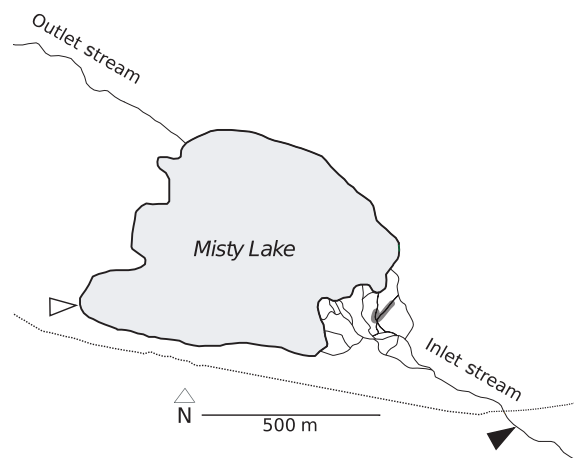

Fig. 1 Location of sampling sites indicated with white (lake) and black (stream) triangles and dark grey shading (contact zone). The dotted line indicates Highway 19.

16 May 2009, we intensively trapped fish in this location. Young-of-the-year and gravid females were immediately released and were not included in the study. We used a Nikon D100 digital camera to photograph the left side of unanaesthetized fish placed on a 1-cm grid for geometric morphometric quantification of body shape. All fish were then immediately released. Analyses of the photographs (details below) revealed that the fish at this location were morphologically intermediate between lake and stream fish, which thus motivated the rest of this study.

Our next sampling period was 14–18 July 2014. Minnow traps were used to sample sticklebacks from the lake, the stream and the contact zone. The lake sample (50°36′19″N, 127°16′9″W) was taken 850 m distant from the lake edge of the contact zone, and the stream sample (50°36′8″N, 127°15′8″W) was taken 600-m upstream from the stream edge of the contact zone (Fig. 1). The contact zone sample was taken at the same location as in 2009 and spanned a linear distance of approximately 60 m. Young-of-the-year and gravid females were immediately released and were not included in the study. For all other fish, we clipped the left pelvic spine to obtain tissue for genetic analysis and to mark fish for mark–recapture analysis. For a subset of these fish, we photographed their left side (unanaesthetized) on a 1-cm grid with a Canon G11 digital camera. Fish that were recaptured during this initial sampling period (i.e. already had a left-side pelvic clip) were noted and released, but a second clip or photograph was not taken. Immediately after processing, all fish were returned to the location from which they were captured.

Our final sampling period took place on 10–13 May 2015 at the same contact zone location as in 2014. During this sampling period, we clipped the right pelvic

spine and photographed the left side of all captured fish using the same methods as in 2014. Fish that had a left-side clip were noted as '2015 recaptures' and processed as normal (i.e. clipped on right side and photographed). Pelvic spine clips remain clearly visible over more than a year, and this marking approach has been used in previous work (Hagen, 1967; Reimchen, 1992; Hendry *et al.*, 2002). Fish that were recaptured during the sampling period (i.e. already had a right-side clip, with or without a left-side clip) were recorded, but a new spine clip or photograph was not taken.

We used geometric morphometrics to obtain body-shape variables for each photographed fish: 50 from the lake, 50 from the stream and 572 from the contact zone across all years. We used the R package geomorph (Adams & Otárola-Castillo, 2013) to digitize 14 landmarks (Fig. 2) previously used on sticklebacks under similar conditions: that is, live and unanaesthetized (Rolshausen *et al.*, 2015). These landmarks were aligned using a generalized Procrustes analysis (GPA) to obtain 28 aligned Procrustes residuals. These residuals were then used in a principal components analysis (PCA) to summarize and visualize the shape variation. The third principal component (PC3) clearly described body depth variation (see Results), a key external trait that typically discriminates lake and stream sticklebacks (e.g. Reimchen *et al.*, 1985; Aguirre, 2009; Berner *et al.*, 2009; Kaeuffer *et al.*, 2012). To evaluate whether our samples revealed similar patterns – and for the relative position of contact zone fish – we analysed PC3 in a two-factor ANOVA with habitat (lake, stream, contact zone), year and the interaction as fixed effects, followed by Tukey's tests.

We next used the first 24 nonzero PCs (i.e. those that explained < 0.01% of the total variation) to perform a linear discriminant analysis (LDA) on the pure lake and stream fish (i.e. fish from the lake and stream sites), thus obtaining a morphological axis (LD1) that maximally separates lake and stream fish. Leave-one-out cross-validation was used to confirm the success of this separation in our data set. We then generated scores for each of the contact zone fish on this lake vs. stream LD1 axis. The morphological basis (i.e. dependence on body depth) of this axis was examined by calculating the Pearson's product–moment correlation between LD1 scores and PC3 scores for the contact zone fish. These scores were then compared between contact zone
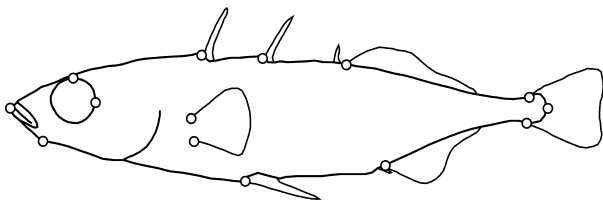
fish and pure-type fish (lake and stream) in a Kruskal–Wallis rank-sum test followed by Mann–Whitney *U*-tests (LD1 had a non-Gaussian distribution in some samples). We also used the maximum *a posteriori* probability to classify fish as lake or stream based on their LD1 scores, in order to determine whether fish in the contact zone were more lake-like or more stream-like.

We used the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) to extract DNA from the spine clips of 29 lake and 29 stream fish. These samples provided a genetic reference sample for pure lake and pure stream fish, which have previously been shown to be very genetically distinct in the Misty system, indicating restricted gene flow: $F_{ST} = 0.129–0.157$ (Hendry *et al.*, 2002), mean $F_{ST} = 0.126$ (Moore *et al.*, 2007), mean neutral $F_{ST} = 0.121$ and mean selected $F_{ST} = 0.275$ (Kaeuffer *et al.*, 2012). We also extracted DNA from (i) all 17 fish that had been marked in 2014 and recaptured in 2015 ('2015 recaptures') and (ii) a random sample of 39 fish captured in 2014, which provided appropriate power to detect differences from the 2015 recaptures.

Extracted DNA was amplified using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany) and following the manufacturer's protocol in a multiplexed PCR for the microsatellite loci STN321, STN246, STN232 and STN168 (Peichel *et al.*, 2001). These specific loci were chosen because previous work had shown that they have nonoverlapping allele sizes between lake and stream fish from the Misty system and thus efficiently differentiate between the two populations (Kaeuffer *et al.*, 2012). Indeed, with only these four loci, we had an excellent ability to do so in our samples (see Results). The PCR products were sized on an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA), and peaks were called on Geneious 8.1.6 (Biomatters, San Francisco, CA, USA).

We first analysed the allele scores for all fish together in the program STRUCTURE (Pritchard *et al.*, 2000). To determine the most likely number of genetic clusters (*K*), we ran five simulations each of *K* = 1–5 with a 25 000 repetition burn-in period followed by 300 000 further repetitions. We used correlated allele frequencies and the locprior model (locations were lake, stream or contact zone), although similar results (not shown) were obtained when not using locprior. We then used Structure Harvester (Earl & vonHoldt, 2012) to determine the most likely *K* value for our data based on the Evanno *et al.* (2005) criteria. Having established that the most likely number of clusters (*K*) was two (see Results), we ran another simulation with *K* = 2 and the same model parameters as before to determine, for each individual, the proportion of ancestry from the lake cluster (q_L) and thus also the stream cluster ($1-q_L$). The q_L values were then used to calculate a hybridity index for each individual as $h = 0.5-|0.5-q_L|$, which ranges from 0 for pure types (i.e. pure lake or pure

Fig. 2 Landmarks used for geometric morphometrics.

stream) to 0.5 for F1 hybrids (Gow *et al.*, 2007). Due to the low number of markers used to calculate q_L, we simulated F1, F2 (F1 × F1), lake backcross (F1 × lake) and stream backcross (F1 × stream) hybrids to determine the expected range of q_L values for fish with those genetic backgrounds (see Appendix S1). We also simulated pure-type crosses (i.e. stream × stream and lake × lake) to approximate variation in q_L for those populations. Finally, to determine whether individuals that were intermediate genetically were also intermediate morphologically, we calculated the Pearson's product–moment correlation between LD1 scores and q_L values for the contact zone fish for which we had both types of data.

We tested for selection against hybrids in several nonparametric tests. First, we used a one-tailed Mann–Whitney *U*-test to examine whether mean *h* decreased from the random sample of 39 contact zone fish marked in 2014 to the 17 fish recaptured in 2015. Second, we used a two-tailed Mann–Whitney *U*-test to determine whether q_L differed between these two samples. Finally, we used a two-tailed Mann–Whitney *U*-test to see whether morphological LD1 scores differed between the 2014 random sample and the 2015 recaptures. This included all fish that had been photographed even if they had not been genotyped.

Results

In the contact zone, we caught 148 fish in 2009, 360 fish in 2014 and 211 fish in 2015. In this last sample, 17 fish were recaptures from 2014. The between-year recapture rate in the contact zone between 2014 and 2015 was thus 4.7%. We suggest that these recaptured fish likely included nearly all of the marked 2014 fish that were still alive and present in the contact zone in 2015. First, the recapture of 2014-marked fish in 2015 decreased from 10.1% on the first day (eight recaptures/79 total fish) to 2.9% on the last day (one new recapture/35 total fish). Second, the within-season recapture rate in 2015 (fish captured, released and recaptured in 2015) increased from 2.5% on the first day (two recaptures/79 total) to 28.6% on the last day (10 recaptures/35 total). Consequently, further sampling in 2015 would not have resulted in a significant number of additional 2015 recaptures and would instead have been composed mostly of within-season recaptures. Similarly, we could not have marked many additional fish in 2014 because capture rates of new fish declined dramatically during our sampling period (from 175 unique fish captured the first day to 13 unique fish on the fifth and final day). In short, the contact zone population is rather small; hence, our samples, while modest in absolute number, are likely a substantial proportion of the population of interest.

The first two PCs from the PCA on Procrustes residuals described shape changes that were dominated by

bending and roll of the fish, which results from taking photographs of live fish in the field. However, the third PC, which accounted for 12.7% of the total shape variation, predominantly described variation in body depth (Fig. 3), which previous studies of the Misty system have shown to be genetically based (e.g. Sharpe *et al.*, 2008; Berner *et al.*, 2011; Oke *et al.*, 2016) and associated with correlates of fitness such as survival (e.g. Rolshausen *et al.*, 2015). Furthermore, PC3 reflected body depth differences along the entire body length and is therefore a more complete measure of body depth variation than a single univariate measurement. Like those previous studies, we found that body depth (PC3) differed strongly between the three habitats ($F_{2,667} = 71.156$, $P < 0.001$, $\eta^2 = 0.189$). *Post hoc* Tukey's tests showed that the contact zone fish were intermediate to, and significantly different from, both pure types (lake contact: $P = 0.002$, stream contact: $P < 0.001$).

The LDA effectively discriminated between lake and stream fish; leave-one-out cross-validation resulted in a misclassification rate of only 3% (one lake fish misclassified as a stream fish and two stream fish misclassified as lake fish). LD1 was strongly correlated with PC3 for the contact zone fish ($r = 0.66$, $t_{570} = 21.099$, $P < 0.001$), suggesting that body depth was the main aspect by which fish were discriminated on LD1. The contact zone fish were intermediate to, and different from, both lake (Mann–Whitney $U = 22\,566$,
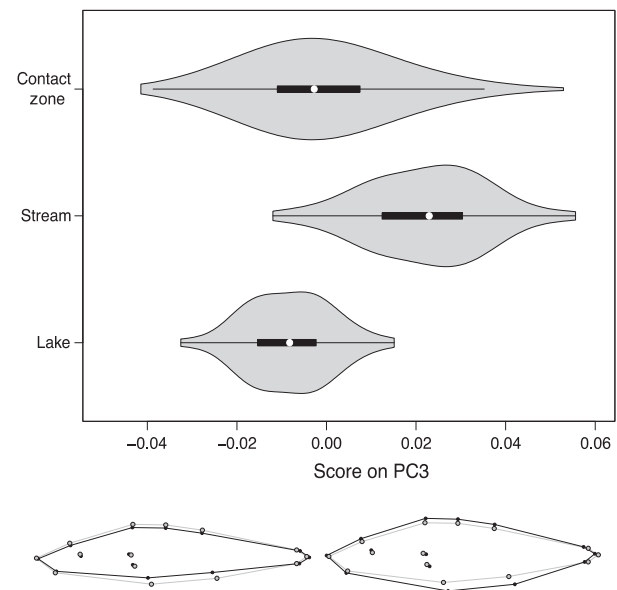

Fig. 3 Violin plots for scores on PC3 for all photographed fish. For each group, white points represent the median, thick black lines the interquartile range, thin black lines 1.5 times the interquartile range, and grey area the kernel density estimation of the data distribution. Deformation grids along the *x*-axis display the change in shape from the mean (grey outlines) to the most extreme individuals on PC3 (black outlines).

$P < 0.001$) and stream fish (Mann–Whitney $U = 1630$, $P < 0.001$) on LD1 (Fig. 4). Although intermediate, the contact zone fish were more often lake-like than stream-like, with the maximum *a posteriori* probability for 405 being to the lake and only 167 being to the stream.

Structure Harvester identified the most probable number of genetic clusters as two. Using $K = 2$, the lake
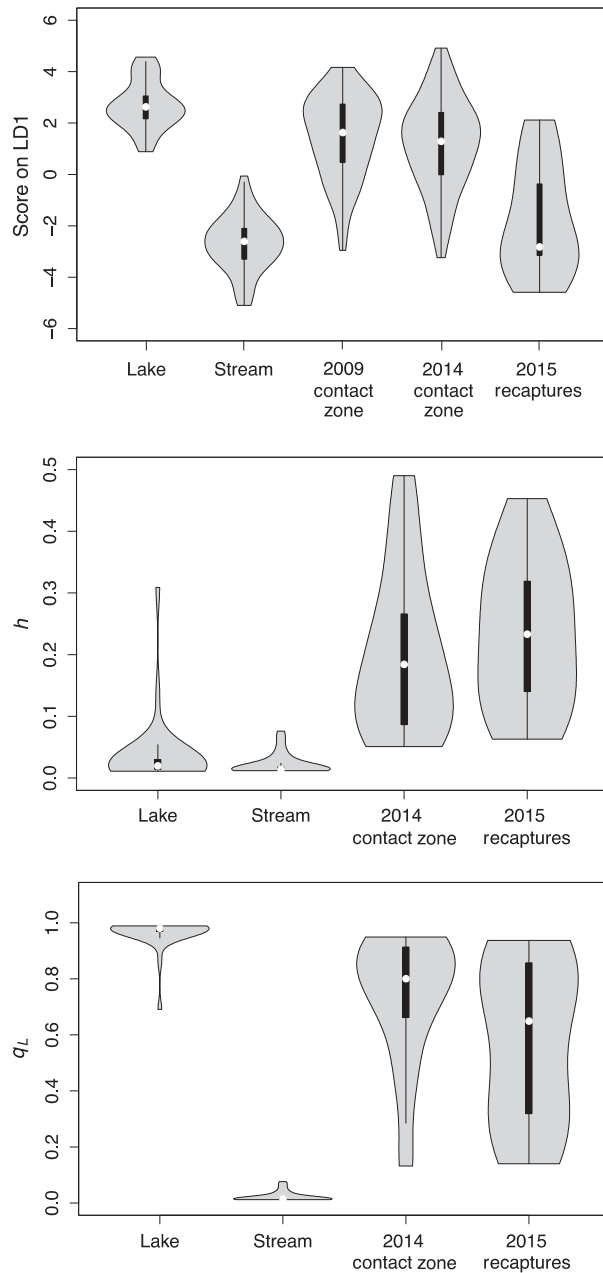

Fig. 4 Violin plots for LD1 scores (top panel), *h* values (middle panel), q_L values (bottom panel) for each sample group. Plot characteristics as in Fig. 3.

and stream populations were highly differentiated from each other, with only two fish having < 90% ancestry from their respective clusters (Fig. 5). Both of these fish, which had 86.4% and 69.1% lake ancestry, were collected from the lake. The contact zone had a mixture of nearly pure types and apparent hybrids (including backcrosses), with per cent ancestry from the lake cluster (q_L) ranging from 13.2% to 94.9%. Mean lake ancestry in the contact zone was 68.2%, indicating again that contact zone fish were on average more lake-like than stream-like. Ranges of q_L values for simulated hybrids were 26.3–66.1% for F1s, 11–82.1% for F2s, 38.4–95.6% for lake backcrosses and 7.6–66.6% for stream backcrosses (Fig. S1); all of the contact zone fish therefore had q_L values within the expected range for hybrids. Furthermore, the range of q_L values for the simulated pure types was 91.1–99.0% for pure lake and 0.2–1.7% for pure stream; 28 of the 39 contact zone fish therefore had q_L values outside those ranges. Hybridity (*h*) in the contact zone fish ranged from 0.05 to 0.49, with a mean value of 0.22. By contrast, mean hybridity in the lake was $h = 0.03$, and in the stream, it was $h = 0.02$ (Fig. 4). The positions of contact zone fish on the lake–stream morphological axis (LD1) were correlated with their positions on the lake–stream genetic axis (q_L) ($r = -0.49$, $t_{51} = -3.998$, $P = 0.0002$) (Fig. 6). Thus, hybrids (inferred genetically) were indeed morphologically intermediate, and either type of data was quite predictive of the other type of data.

Hybridity tended to increase and q_L to decrease between the random sample of 2014 contact zone fish and those recaptured in 2015 ($W = 269.5$, $P = 0.1365$; $W = 434.5$, $P = 0.068$, respectively). These results suggest the possibility of higher survival for hybrids and those fish with a higher proportion of stream ancestry ($1-q_L$) (Fig. 4). This last conclusion was further supported by the significant increase in LD1 scores (towards stream-like morphology) between the 2014 random sample and the 2014 fish that were recaptured in 2015 ($W = 570$, $P < 0.001$) (Fig. 4). For LD1 scores, some 2015 recaptures had scores outside the range of the 2014 marked sample, likely due to the fact that not all fish were photographed in 2014.

Discussion

Our genetic and morphological data show that the Misty lake–stream contact zone has a mixture of almost pure lake and stream fish and also intermediate individuals. We infer that some of these intermediate fish are hybrids, which indicates that lake and stream fish are successfully mating in nature where they come into contact: that is, assortative mating appears incomplete. However, hybridity did not decrease over a year of life, suggesting that selection against hybrids in general is not occurring (but see alternatives below). Although generally intermediate, fish in the contact zone tended
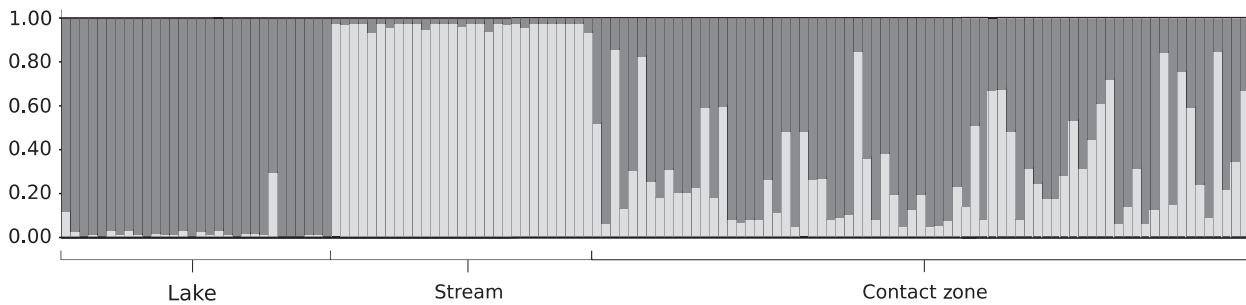
Fig. 5 STRUCTURE plot showing proportion ancestry in each of two clusters (dark grey and light grey) for lake, stream and contact zone fish.
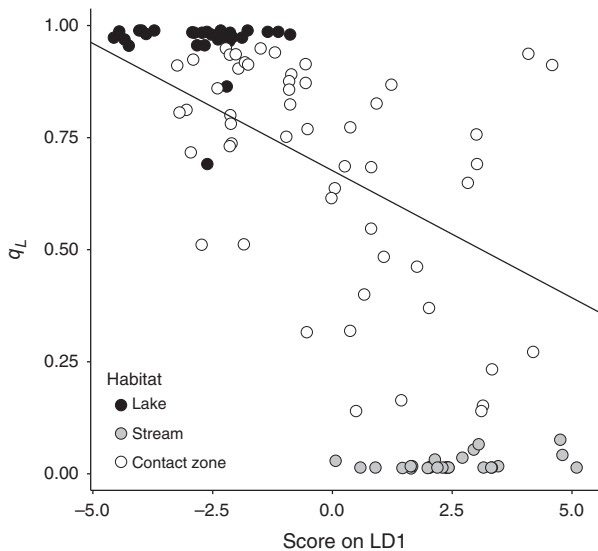

Fig. 6 Correlation between scores on LD1 and q_L values. Correlation line calculated on contact zone fish only.

to be more lake-like than stream-like in terms of both morphology and genetics. However, this tendency decreased over the course of 1 year, suggesting selection against more lake-like individuals.

Interbreeding

The existence of hybrids in the contact zone indicates frequent successful interbreeding between lake and stream fish. This finding echoes previous laboratory-based studies that reported no evidence of mating isolation between these populations (Raeymaekers *et al.*, 2010; Räsänen *et al.*, 2012). We do not know the actual frequency of mating between the two ecotypes, and so we cannot address whether some partial assortative mating might be present – but it does not appear to be a substantial reproductive barrier. This result contrasts with work on other stickleback systems that does show positive assortative mating between ecotypes (Rundle *et al.*, 2000; McKinnon *et al.*, 2004), and suggests that

adaptation to lake and stream environments may not lead to mating isolation as a by-product of such adaptation. One likely candidate for these different results across systems relates to the differential potential for the evolution of reinforcement. First, the lack of selection against lake–stream hybrids demonstrated here (see also Raeymaekers *et al.*, 2010) might mean that positive assortative mating is not under positive selection. Second, the contact zone is quite narrow in relation to the greater lake and stream environments such that any selection for reinforcement might be swamped by movement from the adjacent gene pools that do not experience such selection.

Hybrid selection

We did not find selection against hybrids overall in the contact zone, which again contrasts with studies of other stickleback systems arguing for ecologically based selection against between-ecotype hybrids in nature (Vamosi *et al.*, 2000; Jones *et al.*, 2006; Gow *et al.*, 2007). Several factors might explain this result. First, our sample sizes were quite low (because the total number of fish was low) and although we believe we caught most of the marked fish still present in the area where they had been released in 2014, it is possible that others may have migrated to other areas of the contact zone or into the lake/stream. However, the trend was for hybrids to increase, not decrease, and there is very little evidence of admixture outside of the contact zone, as would be expected if a substantial percentage were leaving the recapture area. Second, we considered selection only on individuals that were at least 1 year of age, whereas selection against hybrids might well occur earlier, as has been found for benthic–limnetic hybrids (Vamosi *et al.*, 2000; Gow *et al.*, 2007; but see Taylor *et al.*, 2012). Third, our genetic data and sample sizes were not sufficient to partition hybrids into different classes (e.g. F1, F2 and backcrosses). If selection varies across these classes, we may be failing to detect stronger selection acting on some classes because it is averaged with potentially weaker selection in other classes (Arnold & Hodges, 1995).

Fitness differences between hybrid classes could be due to the breakdown or creation of genetic interactions influencing the trait under selection (Czesak *et al.*, 2004; Fritz *et al.*, 2006; Fuller, 2008). Indeed, we found an upwards shift in *h* values between 2014 and recaptured 2015 fish (Fig. 4), which may reflect selection against 'deeper' (i.e. backcross) hybrids in favour of more recent hybrids. Fourth, the contact zone might be ecologically intermediate between the lake and the stream environments, in which case intermediate phenotypes actually might be favoured (Coyne & Orr, 2004; Rundle & Nosil, 2005; Taylor *et al.*, 2012). This would suggest that the contact zone is maintained by ecological selection against parental migrants (ecotonal hybrid zone) as opposed to selection against hybrids (tension zone) (Rolán-Alvarez *et al.*, 1997; Dorken & Pannell, 2007).

Following this last point, very few hybrids were detected outside of the contact zone, and we found no evidence of appreciable admixture in the stream and only very slight admixture in the lake. This pattern could reflect three phenomena. First, the number of hybrids generated in the contact zone could be a very small proportion of the lake and stream populations. Therefore, even if they move freely to and survive well in the lake or stream, they would have a small chance of being detected and contribute little to gene flow. Second, hybrids might rarely move into other environments. For instance, habitat preference has been demonstrated in another lake–stream pair and is due in part to differences in rheotactic response (Bolnick *et al.*, 2009; Jiang *et al.*, 2015). Thus, testing the rheotactic responses and habitat preferences of the contact zone fish would be a valuable future experiment. Finally, hybrids might be selected against outside of the contact zone where their intermediate morphology (confirmed to be genetically based: Berner *et al.*, 2011) is poorly suited for either environment. Such a result would be concordant with evidence that F1 hybrids have lower survival than native stream fish in stream field enclosures in Switzerland (Moser *et al.*, 2016), although further tests in the lake environment are needed to determine whether this effect is ecologically dependent. Furthermore, the slightly higher level of admixture in the lake could suggest that selection against hybrids is stronger in the stream than it is in the lake. This scenario is supported by the predictions made by Berner *et al.* (2011) that selection against hybrids should be stronger in the stream than in the lake because hybrids tend to be more lake-like in body depth. Further work will be needed to disentangle these possibilities.

Selection against lake-like individuals

Selection against migrants is thought to be a very important reproductive barrier in young species pairs undergoing ecological divergence (Hendry, 2004; Nosil *et al.*, 2005; Thibert-Plante & Hendry, 2009). A previous experimental one-way transplant in the Misty system suggested strong selection against lake fish in the stream (Hendry *et al.*, 2002), but reciprocal enclosure transplants yielded more ambiguous outcomes (Hendry *et al.*, 2002; Räsänen & Hendry, 2014). The current results provide an additional perspective. In particular, individuals in the contact zone tended to be more lake-like, both morphologically and genetically, perhaps because the lake population is very large and stream fish tend not to move downstream (Hendry *et al.*, 2002; Bolnick *et al.*, 2009; Jiang *et al.*, 2015). Interestingly, however, this lake-like bias decreased over a 1-year period, suggesting that more lake-like individuals had lower survival in the contact zone.

This selection against lake-like fish in the contact zone could reflect the intersection of two phenomena. First, laboratory studies have shown that Misty hybrids (F1s, F2s and backcrosses) tend to be more lake-like than stream-like in body depth due to genetic dominance (Berner *et al.*, 2011). Second, we postulate that the contact zone is more stream-like than lake-like in its environmental features. As noted earlier, the contact zone is a marshy environment cut with narrow, low-flow channels and hence having a relative scarcity of open water. Sustained swimming in open water while foraging on zooplankton is thought to be the main agent of selection for streamlined, shallow bodies in lake fish (Berner *et al.*, 2008; Hendry *et al.*, 2011). Thus, we propose that lake-like individuals are experiencing stronger negative selection in the contact zone than are stream-like individuals, because the environment is more stream-like. The ideal experiment to address these suppositions would be to track the fate of hybrids transplanted into the stream (e.g. Moser *et al.*, 2016) and lake environments, which – owing to logistical and ethical considerations – would have to take place in experimental enclosures. Finally, it is possible that the decrease in lake-like characteristics reflects random year-to-year or site-to-site variation in survival; repeating this experiment over several years and sites would be optimal in assessing these possibilities.

Multiple interacting reproductive barriers

When the results of this study are taken together with previous work on reproductive isolation between lake and stream sticklebacks (e.g. Hendry *et al.*, 2002; Bolnick *et al.*, 2009; Räsänen *et al.*, 2012; Räsänen & Hendry, 2014; Jiang *et al.*, 2015; Marques *et al.*, 2016; Moser *et al.*, 2016), it becomes clear that a combination of several (mostly weak and asymmetric) barriers to reproduction are working in concert to restrict gene flow to sometimes very low levels. Analogous patterns have been found in some other well-studied systems. For example, nine different barriers each make different (sometimes opposing) contributions to reproductive isolation in monkeyflowers (*Mimulus* spp.) that ultimately

combine to generate total reproductive isolation of between 0.997 and 0.999 (Ramsey *et al.*, 2003). Similar outcomes have been documented in other plants (e.g. Chari & Wilson, 2001; Husband & Sabara, 2004; Kay, 2006) and in invertebrates (Nosil *et al.*, 2003; Nosil, 2007), but are rare overall (Rundle & Nosil, 2005). Future work in sticklebacks (and in general) would benefit from an approach that concurrently evaluates many potential barriers in a single system.

Acknowledgments

References

Adams, D.C. & Otárola-Castillo, E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* **4**: 393–399.

Aguirre, W.E. 2009. Microgeographical diversification of three-spine stickleback: body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. *Biol. J. Linn. Soc.* **98**: 139–151.

Alexandrino, J., Baird, S.J.E., Lawson, L., Macey, J.R., Moritz, C. & Wake, D.B. 2005. Strong selection against hybrids at a hybrid zone in the *Ensatina* ring species complex and its evolutionary implications. *Evolution* **59**: 1334–1347.

Arnold, M.L. & Hodges, S.A. 1995. Are natural hybrids fit or unfit relative to their parents?. *Trends Ecol. Evol.* **10**: 67–71.

Behm, J.E., Ives, A.R. & Boughman, J.W. 2010. Breakdown in postmating isolation and the collapse of a species pair through hybridization. *Am. Nat.* **175**: 11–26.

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

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

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

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

Boughman, J.W., Rundle, H.D. & Schluter, D. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution* **59**: 361–373.

Castillo, D.M., Burger, M.K., Lively, C.M. & Delph, L.F. 2015. Experimental evolution: assortative mating and sexual selection, independent of local adaptation, lead to reproductive isolation in the nematode *Caenorhabditis remanei*. *Evolution* **69**: 3141–3155.

Chari, J. & Wilson, P. 2001. Factors limiting hybridization between *Penstemon spectabilis* and *Penstemon centranthifolius*. *Can. J. Bot.* **79**: 1439–1448.

Coyne, J.A. & Orr, H.A. 2004. *Speciation*. Sinauer Associates, Inc. Sunderland, MA.

Czesak, M., Knee, M., Gale, R., Bodach, S. & Fritz, R. 2004. Genetic architecture of resistance to aphids and mites in a willow hybrid system. *Heredity* **93**: 619–626.

Dorken, M. & Pannell, J. 2007. The maintenance of hybrid zones across a disturbance gradient. *Heredity* **99**: 89–101.

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**: 359–361.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620.

Faria, R., Renaut, S., Galindo, J., Pinho, C., Melo-Ferreira, J., Melo, M. *et al.* 2014. Advances in ecological speciation: an integrative approach. *Mol. Ecol.* **23**: 513–521.

Fritz, R.S., Hochwender, C.G., Albrectsen, B.R. & Czesak, M.E. 2006. Fitness and genetic architecture of parent and hybrid willows in common gardens. *Evolution* **60**: 1215–1227.

Fuller, R.C. 2008. Genetic incompatibilities in killifish and the role of environment. *Evolution* **62**: 3056–3068.

Funk, D.J., Nosil, P. & Etges, W.J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proc. Natl. Acad. Sci. USA* **103**: 3209–3213.

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

Hagen, D.W. 1967. Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). *J. Fish. Res. Board Can.* **24**: 1637–1692.

Hanson, D., Barrett, R. & Hendry, A. 2016. Testing for parallel allochronic isolation in lake–stream stickleback. *J. Evol. Biol.* **29**: 47–57.

Hendry, A.P. 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evol. Ecol. Res.* **6**: 1219–1236.

Hendry, A.P. 2009. Ecological speciation! Or the lack thereof? *Can. J. Fish Aquat. Sci.* **66**: 1383–1398.

Hendry, A.P. 2016. Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *J. Hered.* **107**: 25–41.

Hendry, A.P. & Taylor, E.B. 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., Taylor, E.B. & McPhail, J.D. 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., Bolnick, D.I., Berner, D. & Peichel, C.L. 2009. Along the speciation continuum in sticklebacks. *J. Fish Biol.* **75**: 2000–2036.

Hendry, A., Hudson, K., Walker, J., Räsänen, K. & Chapman, L. 2011. Genetic divergence in morphology–performance

mapping between Misty Lake and inlet stickleback. *J. Evol. Biol.* **24**: 23–35.

Honma, Y. & Tamura, E. 1984. Anatomical and behavioral differences among threespine sticklebacks: the marine form, the landlocked form and their hybrids. *Acta Zool.* **65**: 79–87.

Hopkins, R., Guerrero, R.F., Rausher, M.D. & Kirkpatrick, M. 2014. Strong reinforcing selection in a Texas wildflower. *Curr. Biol.* **24**: 1995–1999.

Husband, B.C. & Sabara, H.A. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytol.* **161**: 703–713.

Jiang, Y., Torrance, L., Peichel, C.L. & Bolnick, D.I. 2015. Differences in rheotactic responses contribute to divergent habitat use between parapatric lake and stream threespine stickleback. *Evolution* **69**: 2517–2524.

Jones, F.C., Brown, C., Pemberton, J.M. & Braithwaite, V.A. 2006. Reproductive isolation in a threespine stickleback hybrid zone. *J. Evol. Biol.* **19**: 1531–1544.

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

Kay, K.M. 2006. Reproductive isolation between two closely related hummingbird pollinated neotropical gingers. *Evolution* **60**: 538–552.

Langerhans, R.B., Gifford, M.E. & Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. *Evolution* **61**: 2056–2074.

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

Lindtke, D., Gompert, Z., Lexer, C. & Buerkle, C.A. 2014. Unexpected ancestry of *Populus* seedlings from a hybrid zone implies a large role for postzygotic selection in the maintenance of species. *Mol. Ecol.* **23**: 4316–4330.

Lowry, D.B., Rockwood, R.C. & Willis, J.H. 2008. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution* **62**: 2196–2214.

Marques, D.A., Lucek, K., Meier, J.I., Mwaiko, S., Wagner, C.E., Excoffier, L. *et al.* 2016. Genomics of rapid incipient speciation in sympatric threespine stickleback. *PLoS Genet.* **12**: e1005887.

McKinnon, J.S. & Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *Trends Ecol. Evol.* **17**: 480–488.

McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L. *et al.* 2004. Evidence for ecology's role in speciation. *Nature* **429**: 294–298.

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

Moser, D., Frey, A. & Berner, D. 2016. Fitness differences between parapatric lake and stream stickleback revealed by a field transplant. *J. Evol. Biol.* **29**: 711–719.

Nosil, P. 2007. Divergent host plant adaptation and reproductive isolation between ecotypes of *Timema cristinae* walking sticks. *Am. Nat.* **169**: 151–162.

Nosil, P. 2012. *Ecological Speciation*. Oxford University Press, Oxford, UK.

Nosil, P., Crespi, B.J. & Sandoval, C.P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature* **417**: 440–443.

Nosil, P., Crespi, B. & Sandoval, C. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **270**: 1911–1918.

Nosil, P., Vines, T.H. & Funk, D.J. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**: 705–719.

Oke, K., Bukhari, M., Kaeuffer, R., Rolshausen, G., Räsänen, K., Bolnick, D. *et al.* 2016. Does plasticity enhance or dampen phenotypic parallelism? A test with three lake–stream stickleback pairs. *J. Evol. Biol.* **29**: 126–143.

Peichel, C.L., Nereng, K.S., Ohgi, K.A., Cole, B.L.E., Colosimo, P.F., Buerkle, C.A. *et al.* 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* **414**: 901–905.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Raeymaekers, J.A.M., Boisjoly, M., Delaire, L., Berner, D., Räsänen, K. & Hendry, A.P. 2010. Testing for mating isolation between ecotypes: laboratory experiments with lake, stream and hybrid stickleback. *J. Evol. Biol.* **23**: 2694–2708.

Ramsey, J., Bradshaw, H.D. & Schemske, D.W. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**: 1520–1534.

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

Räsänen, K., Delcourt, M., Chapman, L.J. & Hendry, A.P. 2012. Divergent selection and then what not: the conundrum of missing reproductive isolation in Misty lake and stream stickleback. *Int. J. Ecol.* **2012**: 902438.

Ravinet, M., Prodöhl, P.A. & Harrod, C. 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. 1992. Extended longevity in a large-bodied stickleback, *Gasterosteus*, population. *Can. Field-Nat.* **106**: 122–125.

Reimchen, T.E., Stinson, E.M. & Nelson, J.S. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Can. J. Zool.* **63**: 2944–2951.

Roesti, M., Kueng, B., Moser, D. & Berner, D. 2015. The genomics of ecological vicariance in threespine stickleback fish. *Nat. Commun.* **6**: 1–14.

Rolán-Alvarez, E., Johannesson, K. & Erlandsson, J. 1997. The maintenance of a cline in the marine snail *Littorina saxatilis*: the role of home site advantage and hybrid fitness. *Evolution* **51**: 1838–1847.

Rolshausen, G., Muttalib, S., Kaeuffer, R., Oke, K.B., Hanson, D. & Hendry, A.P. 2015. When maladaptive gene flow does not increase selection. *Evolution* **69**: 2289–2302.

Rundell, R.J. & Price, T.D. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* **24**: 394–399.

Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecol. Lett.* **8**: 336–352.

Rundle, H.D., Nagel, L., Boughman, J.W. & Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**: 306–308.

Schluter, D. 2000. Ecological character displacement in adaptive radiation. *Am. Nat.* **156**: S4–S16.

Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* **16**: 372–380.

Schwartz, A.K., Weese, D.J., Bentzen, P., Kinnison, M.T. & Hendry, A.P. 2010. Both geography and ecology contribute to mating isolation in guppies. *PLoS One* **5**: e15659.

Shafer, A. & Wolf, J.B. 2013. Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecol. Lett.* **16**: 940–950.

Sharpe, D.M., Räsänen, K., Berner, D. & Hendry, A.P. 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.

Shurtliff, Q.R., Murphy, P.J. & Matocq, M.D. 2014. Ecological segregation in a small mammal hybrid zone: habitat-specific mating opportunities and selection against hybrids restrict gene flow on a fine spatial scale. *Evolution* **68**: 729–742.

Svensson, E.I. 2012. Non-ecological speciation, niche conservatism and thermal adaptation: how are they connected? *Org. Divers. Evol.* **12**: 229–240.

Tastard, E., Ferdy, J.B., Burrus, M., Thébaud, C. & Andalo, C. 2012. Patterns of floral colour neighbourhood and their effects on female reproductive success in an *Antirrhinum* hybrid zone. *J. Evol. Biol.* **25**: 388–399.

Taylor, E.B., Gerlinsky, C., Farrell, N. & Gow, J.L. 2012. A test of hybrid growth disadvantage in wild, free-ranging species pairs of threespine stickleback (*Gasterosteus aculeatus*) and its implications for ecological speciation. *Evolution* **66**: 240–251.

Thibert-Plante, X. & Hendry, A. 2009. Five questions on ecological speciation addressed with individual-based simulations. *J. Evol. Biol.* **22**: 109–123.

Thompson, C.E., Taylor, E.B. & McPhail, J.D. 1997. Parallel evolution of lake-stream pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA variation. *Evolution* **51**: 1955–1965.

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

Toro, J., Innes, D. & Thompson, R. 2004. Genetic variation among life-history stages of mussels in a *Mytilus edulis–M. trossulus* hybrid zone. *Mar. Biol.* **145**: 713–725.

Vamosi, S., Hatfield, T. & Schluter, D. 2000. A test of ecological selection against young-of-the-year hybrids of sympatric sticklebacks. *J. Fish Biol.* **57**: 109–121.

Via, S., Bouck, A.C. & Skillman, S. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution* **54**: 1626–1637.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Histograms of q_L values for simulated hybrids, lake, stream, and contact zone samples.
Appendix S1 Methods for hybrid simulations.