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Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake–stream divergence in parapatric Swiss stickleback

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Abstract

The relative importance of ecological selection and geographical isolation in promoting and constraining genetic and phenotypic differentiation among populations is not always obvious. Interacting with divergent selection, restricted opportunity for gene flow may in some cases be as much a cause as a consequence of adaptation, with the latter being a hallmark of ecological speciation. Ecological speciation is well studied in parts of the native range of the three-spined stickleback. Here, we study this process in a recently invaded part of its range. Switzerland was colonized within the past 140 years from at least three different colonization events involving different stickleback lineages. They now occupy diverse habitats, ranging from small streams to the pelagic zone of large lakes. We use replicated systems of parapatric lake and stream populations, some of which trace their origins to different invasive lineages, to ask (i) whether phenotypic divergence occurred among populations inhabiting distinct habitats, (ii) whether trajectories of phenotypic divergence follow predictable parallel patterns and (iii) whether gene flow constrains divergent adaptation or vice versa. We find consistent phenotypic divergence between populations occupying distinct habitats. This involves parallel evolution in several traits with known ecological relevance in independent evolutionary lineages. Adaptive divergence supersedes homogenizing gene flow even at a small spatial scale. We find evidence that adaptive phenotypic divergence places constraints on gene flow over and above that imposed by geographical distance, signalling the early onset of ecological speciation.

Introduction

The role of gene flow in either constraining or facilitating adaptive population divergence and speciation is a long-standing debate (e.g. Slatkin, 1987; Nosil & Crespi, 2004; Räsänen & Hendry, 2008; Abbott *et al.*, 2013). On the one hand, theory suggests that gene flow can impose important constraints on adaptive divergence by homogenizing allele frequencies and preventing the formation of co-adapted gene complexes (Haldane, 1948; Mayr, 1963; Slatkin, 1973, 1987; Endler, 1977; Hendry *et al.*, 2001). As a consequence, gene flow may hamper or completely prevent adaptive divergence and speciation. On the other hand, gene flow can also facilitate diversification by introducing adaptive genetic variation and increasing the adaptive potential of populations overall (Garant *et al.*, 2007; Abbott *et al.*, 2013). Migration can also be nonrandom with regard to environment, and the resulting gene flow may thus also be adaptive (Edelaar

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& Bolnick, 2012), for example due to matching habitat choice, where individuals migrate to an environment that best matches their phenotype (Edelaar *et al.*, 2008; Bolnick *et al.*, 2009). When gene flow is maladaptive, adaptive divergence can impose itself a constraint on gene flow, namely when divergent natural and/or sexual selection cause extrinsic reproductive isolation (Schluter, 2000; Maan & Seehausen, 2011). Understanding the relationship and the balance between adaptive divergence and gene flow is therefore essential to understand the relative importance of selection and geographical isolation during speciation (Mayr, 1963; Endler, 1977; Hendry *et al.*, 2001; Coyne & Orr, 2004; Nosil & Crespi, 2004; Räsänen & Hendry, 2008). Doing so, however, requires studying the very early stages of replicated ecotypic divergence before strong extrinsic (and any intrinsic) reproductive isolation has evolved (Hendry *et al.*, 2000; Shafer & Wolf, 2013).

Adaptive population divergence may be repeated and predictable if the underlying divergent selection regime is comparable, similar genetic variation is present, and if maladaptive gene flow is not too strong (Endler, 1977; Doebeli & Dieckmann, 2003; Räsänen & Hendry, 2008). Indeed, ecological adaptation leads to parallel phenotypic differentiation in ecologically relevant traits in population pairs occupying different ecological contrasts (Schluter, 2000), where selection reduces phenotypic overlap coupled with adaptation to different adaptive peaks (Leimar *et al.*, 2008). Such phenotypic adaptation can occur despite gene flow if selection is sufficiently strong or migration is nonrandom with regard to adaptation as in habitat matching (Edelaar & Bolnick, 2012). Phenotypic divergence of populations can be initiated by ecological specialization and phenotypic plasticity at the individual level (Pfennig *et al.*, 2010) and can itself precede the origin of measurable reproductive isolation.

Ecological speciation in parapatry is often associated with adaptation to different environments and occurs often along environmental gradients (Endler, 1977; Dieckmann *et al.*, 2004; Terai *et al.*, 2006; Ingram, 2011). This has been studied in parapatric three-spined stickleback (*Gasterosteus aculeatus*) lake–stream systems, which mostly evolved after the last glacial maximum (Hagen & Gilbertson, 1972; Gross & Anderson, 1984; Reimchen *et al.*, 1985; Hendry & Taylor, 2004; Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013; but see Berner *et al.*, 2010; Hendry *et al.*, 2013). Stream populations in these systems often exhibit morphological features more conducive to feeding on benthic river invertebrates, whereas lake populations fall along a continuum between two possible ecotypes, one feeding on benthic invertebrates associated with the littoral zone and the other feeding on plankton in the limnetic zone of lakes. Although the divergence of stickleback ecotypes has in some instances occurred despite a high potential for gene flow (Schluter & McPhail, 1992; Rundle *et al.*, 2000; Hendry *et al.*, 2001; Berner *et al.*, 2009; Roesti *et al.*, 2012), in others, divergence seems constrained by gene flow due to potential genetic constraints (Hendry *et al.*, 2002; Berner *et al.*, 2010) or the time since divergence (Berner *et al.*, 2010; Hendry *et al.*, 2013).

Most evidence for the role of divergent environments in promoting adaptive divergence and ecological speciation, however, comes from long-established populations, where the processes that underlie adaptive divergence are difficult to infer. In particular, ecological speciation has been studied in evolutionarily young systems, such as cichlid fishes in Nicaraguan lakes (Elmer *et al.*, 2010) and Lake Victoria (Seehausen *et al.*, 2008) or cases of post-glacial colonization and diversification of freshwater fishes in north temperate lakes (e.g. Sandlund *et al.*, 1992; Schluter, 2000; Bernatchez *et al.*, 2010; Hudson *et al.*, 2011) and parapatric lake–stream systems in stickleback (Hagen & Gilbertson, 1972; Reimchen *et al.*, 1985; Hendry & Taylor, 2004; Berner *et al.*, 2008, 2009; Kaeuffer *et al.*, 2012). Accrued empirical evidence suggests that ecological divergence can sometimes occur rapidly over just a few generations (e.g. Hendry *et al.*, 2000; Eroukhmanoff *et al.*, 2009; Leaver & Reimchen, 2012; see Hendry *et al.*, 2007 for a review). Rapid ecological divergence has also been shown during biological invasions (Hendry *et al.*, 2000; Phillips & Shine, 2006; Westley, 2011), which do provide great opportunities to study the very early stages of adaptive divergence and, in some cases, ecological speciation (Prentis *et al.*, 2008; Yoder *et al.*, 2010; Westley, 2011). Consequently, studying successful invasions with range expansion into several distinct habitat niches associated with phenotypic divergence may help clarify the ecological and genetic constraints that need to be surmounted during the early stages of ecological speciation.

In Switzerland, stickleback were restricted to tributaries of the Rhine near Basel north of the Jura Mountains, being absent from the Swiss midlands until about 1870 (Heller, 1870; Fatio, 1882; Bertin, 1925; Lucek *et al.*, 2010). Following subsequent introductions and the channelization of many Swiss waterways for irrigation (Heller, 1870; Fatio, 1882; Bertin, 1925), stickleback underwent a range expansion and now occur in large parts of the country, occupying a wide range of different habitats, ranging from tiny streams to very large lakes with vast pelagic zones (Lucek *et al.*, 2010). Consequently, they provide an exceptional opportunity to study the replicated parallel initiation of ecotypic differentiation over short evolutionary timescales (~140 generations, Table 1). These historically independent and replicated lake–stream habitat contrasts also provide opportunities to examine the relationship between gene flow and divergence under variable levels of geographi-

Table 1 Characteristics of sampling sites for Swiss sticklebacks used in this study with coordinates and sample sizes used for microsatellite and geometric morphometrics (*N*). The expected heterozygosity (H_E) is based on 17 microsatellites is furthermore indicated. Abbreviations for habitats: L, lake; S, stream; M, mouth of stream near its inflow into the lake. Introduction dates based on historical reports (Lucek *et al.*, 2010) refer to lake systems, rather than to specific sites or habitats. The age of Lake Wohlen, a man-made dam is indicated too.

System	Habitat	N	E	Waterway distance to lake (km)	Altitude above lake (m)	*N*	Introduction	H_E
Constance	L	47°29′08″	9°32′37″	< 0.1	–	30	~1870*	0.551
	S	47°19′33″	9°34′41″	27.1	23	50		0.511
Geneva	L	46°31′02″	6°34′41″	0	–	38	~1870†‡	0.485
	M	46°23′07″	6°51′30″	< 0.2	3	60		0.490
	S	46°12′50″	7°18′53″	61.0	92	35		0.470
Biel	L	47°54′57″	7°11′59″	< 0.1	–	27		0.614
	S	46°58′58″	7°15′07″	16.5	33	36		0.625
Bern	L	46°57′59″	7°21′08″	0	–	33	After 1921§	0.623
	M	46°57′41″	7°22′46″	0.3	1	34		0.605
	S	46°59′30″	7°24′42″	14.6	90	28		0.610
Neuchatel	S1	46°47′31″	6°37′43″	0.3	4	35	~1920‡	0.492
	S2	46°38′30″	6°37′36″	1.1	1	31		0.524

*Heller (1870).
†Fatio (1882).
‡Bertin (1925).
§Construction date of the Lake Wohlen dam.

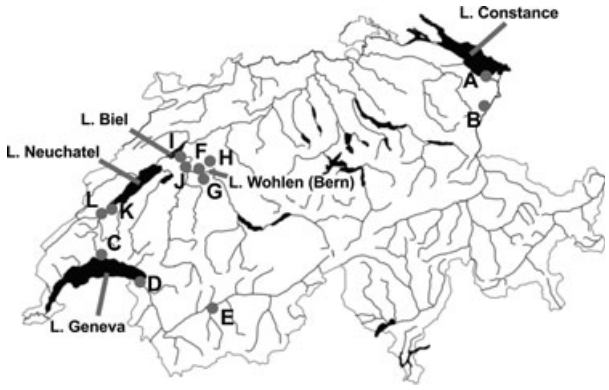

Fig. 1 Stickleback sampling sites used in this study: A – Constance L; B – Constance S; C – Geneva L; D – Geneva M; E – Geneva S; F – Bern L; G – Bern M; H – Bern S; I – Biel L; J – Biel S; K – Neuchatel S1 (near lake); L – Neuchatel S2 (upstream). Sample sites belong to the Rhine drainage (A, B); Rhone drainage (C–E), Aare drainage (F–J) or the Orbe drainage (K, L), respectively (see Table 1 for details; © Wikimedia).

cal isolation. Mitochondrial DNA surveys from populations across the country revealed the colonization of Switzerland by three distant lineages from different parts of Europe (Lucek *et al.*, 2010). The Lake Constance area (Fig. 1) is dominated by East European haplotypes from the Baltic region, whereas the Lake Geneva area is dominated by a lineage typical of the lower Rhone river valley from the Mediterranean drainage. A third presumably native Swiss lineage dominates the Basel region (Lucek *et al.*, 2010). From these presumed native and introduction sites, the three lineages expanded into the Swiss midlands and met in large parts of northern and western Switzerland. In places such as Lakes Neuchatel, Biel and Wohlen (Bern; Fig. 1), populations have a mix of all mitochondrial haplotypes associated with a considerable elevation in haplotypic richness. Nuclear markers (AFLPs) also suggest admixture between the major lineages in these areas, and stickleback from here also display a marked increase in phenotypic diversity and variation (Lucek *et al.*, 2010). The midlands of Switzerland are characterized by many large and deep lakes, some oligotrophic, others meso- and eutrophic, lying in a rich network of streams and canals, which overall leads to extreme habitat contrasts between streams and lakes.

Here, we ask whether the recent range expansion of three-spined stickleback in Switzerland is repeatedly and predictably associated with the onset of ecotypic differentiation between the major habitats and to what extent the associated divergence in phenotypes is predictable. We contrast populations inhabiting three large, deep and oligo- to mesotrophic lakes and their associated streams, and one much smaller, shallower eutrophic lake and its associated streams. Specifically, we assess whether appreciable trait divergence has occurred over this short timescale, whether it is repeatable and whether it is measurably constrained by the opportunity for gene flow. Finally, we evaluate whether adaptive divergence constrains gene flow, that is, whether we can detect signals of the early onset of ecological speciation (Nosil *et al.*, 2009). To address these questions, we investigate variation and diver-

gence at genetic markers, putatively functional phenotypic traits (armour, linear morphology of head and jaws, morphometric shape) and resource use inferred from stable isotopes.

Materials and methods

Sampling sites

We sampled stickleback populations inhabiting ecologically contrasting habitats potentially connected by gene flow in five lake systems of Switzerland: three large natural lakes and associated streams (systems of lakes Constance, Geneva and Biel), one smaller man-made lake (Lake Wohlen, Bern) and its associated streams, and two streams associated with Lake Neuchatel, (Fig. 1; Table 1). In the case of Neuchatel, no lake-dwelling populations could be obtained during our screening of the area. Population abbreviations indicate the name of the lake system from which they were obtained followed by a habitat-dependent indicator (L – lake, S – stream, M – stream mouth). We collected the lake-dwelling sticklebacks on their breeding grounds (i.e. canals adjacent to the lake shore or small stream inlets as well as marinas within the lakes) to obtain adult phenotypes and because the large, deep and oligotrophic Swiss lakes make collecting stickleback in the pelagic where they feed during fall and winter nearly impossible. Here, we classified breeding populations in lake inlets as lake populations when the presence of adults was restricted to the breeding season (e.g. Constance L; Biel L) and as stream resident populations when adults were present year round (e.g. Geneva M; Bern S). Such information was unavailable for the Neuchatel system and as a consequence, we refer to these two collections as stream samples with different distances from the lake (near lake and upstream). In the Geneva and Bern systems, we sampled three sites: a lake site, a stream site very near its outflow to the lake (stream–mouth) and an upstream site. Using hand nets and minnow traps, we collected sticklebacks between April and August 2007 and 2008. Sample sizes varied from 27 to 60 individuals per location (Table 1). We photographed each fish alive in the field in a standardized photo cuvette (10 × 10 × 2.5 cm). To avoid parallax error, we confined the fish to a space barely wider than its body and preventing its movements temporarily using a plastic panel. Fish were then killed with an overdose of anaesthetic MS-222 and preserved in individual tubes with 95% ethanol.

Genetic differentiation

We extracted genomic DNA from fin tissue and genotyped eighteen microsatellite loci, selected from Peichel *et al.* (2001) and located on 15 of 26 linkage groups. Seven of these markers have been shown to be associated with known QTLs for spine lengths, the number of lateral plates and gill rakers (Peichel *et al.*, 2001). For these markers, we predict that, if they are linked to a phenotype under divergent selection, habitat-dependent divergent selection should lead to an increased parapatric genetic differentiation relative to that in neutral markers. A detailed description of each marker together with the PCR and multiplexing protocols is available in the Data S1 supplement.

To evaluate genetic diversity observed in Swiss populations relative to that observed throughout the European range, we compared expected heterozygosities in Swiss samples to those reported from 58 populations sampled from the entire spectrum of other European freshwater habitats similarly genotyped at 18 microsatellite loci (Mäkinen *et al.*, 2006). We measured the pairwise genetic distance between collected samples as F_{ST} and assessed their *P*-values from 10 000 permutations (Meirmans & Van Tienderen, 2004). To quantify the relative importance of lake system vs. habitat nested in lake system in the partitioning of genetic variation, we employed an analysis of molecular variance (AMOVA) using GENODIVE 2.0 (Meirmans & Van Tienderen, 2004). In addition, we generated a genetic tree-like relationship among populations based on their pairwise F_{ST}s using 1000 bootstrapped resampling replicates to assess significance based on a neighbour-joining algorithm implemented in the program PHYLIP 3.69 (Felsenstein, 2012). Finally, we assessed genetic clustering within each lake–stream system, excluding Neuchatel using STRUCTURE 2.3.3 (Falush *et al.*, 2007) based on an admixture model implemented in with 30 000 burnin steps followed by 300 000 MCMC steps. For each system, we took the sampling location as prior information for the clustering due to the low expected level of genetic differentiation given the evolutionary age of the systems (Hubisz *et al.*, 2009).

Phenotypic measurements

We measured sixteen linear traits that are related to feeding ecology, antipredator defence or general body shape and swimming behaviour (Kristjánsson *et al.*, 2002; Mori & Takamura, 2004; Berner *et al.*, 2008; Hendry *et al.*, 2011; and references therein) on each individual to the nearest 0.01 mm using a digital caliper (see Fig. 2 for details). We also counted the total number of gill rakers for each individual and took the mean length of the 2nd to 4th rakers, as counted from the joint of the dorsal arch bone, on the first lower gill arch using a micrometer mounted to a dissection microscope following Berner *et al.* (2008).

As all linear measurements were significantly correlated with standard length (results not shown), we regressed each trait against standard length over all individuals, retaining the residuals. By pooling all systems, allometric information in some populations may

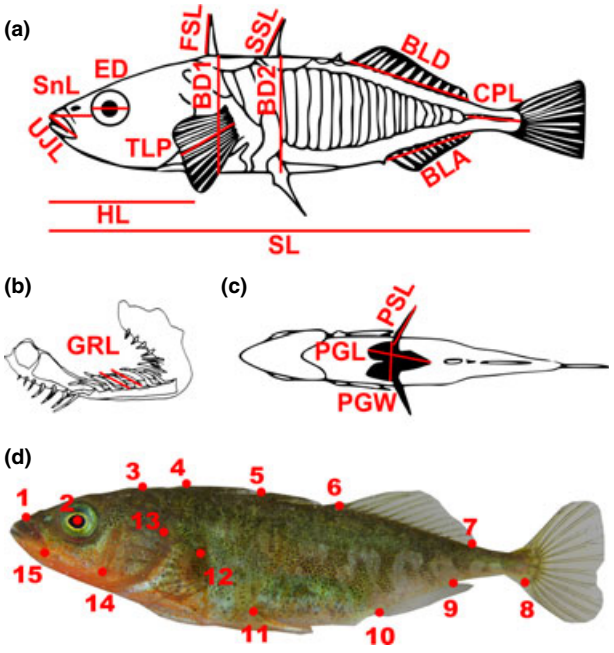

Fig. 2 Summary of all morphological measurements used in this study for linear measurements (a–c), which were either obtained on the left side (a), the gill arch (b) or from the ventral side of each individual as well as geometric morphometric landmarks (d). An Alizarin red-stained individual is shown to highlight the geometric morphometric landmarks used in this study. Linear measurements were as follow: FSL – length of the 1st dorsal spine, SSL – length of the 2nd dorsal spine, PSL – length of the pelvic spine, PGL – length of the pelvic girdle, HL – head length, UJL – upper jaw length, SnL – snout length, SnW – snout width, ED – eye diameter, SL – standard length, PGW – width of the pelvic girdle, BD1 -body depth measured after the 1st dorsal spine, BD2 – body depth measured after the 2nd dorsal spine, CPL – caudal peduncle length, BLA – basal length of the anal fin, BLD – basal length of the dorsal fin and TLP – total length of the pelvic fin. In addition, the length of the 3rd and 4th gill raker was measured. Geometric morphometric landmarks were as follow: 1 – anterior-most point of premaxillary bone, 2 – centre of the eye, 3 – junction of head and dorsal scales. 4 – insertion of the 1st spine, 5 – insertion of the 2nd spine, 6 – anterior end of dorsal fin, 7 – posterior end of dorsal fin, 8 – junction of lower caudal peduncle and tail fin, 9 – posterior end of anal fin, 10 – anterior end of anal fin, 11 – posterior junction of pelvic spine and body, 12 – upper insertion point of pectoral fin, 13 – posterior edge of operculum, 14 – ventral inflexion of pre-opercular bone and 15 – posterior-most point of premaxillary bone.

be retained if the allometric trajectories differ between populations from different study systems. This allows, however, to estimate the system-specific component of phenotypic variation, which is explained by different historical contingencies. Because all individuals are treated the same, the estimates of habitat-dependent parapatric differentiation should reflect the actual degree of divergence. All further analyses based on linear measurements are consequently based on these overall scale-free residuals. We analysed traits either separately or combined using principal component analyses (PCA) based on covariance matrices. PCAs combined either all linear traits or only traits that are linked to antipredator defence (FSL, SSL, PSL, PGL; Fig. 2) or feeding (HL, ED, SnL, UJL, GRL). Especially, the number of gill rakers (GRN) and their length (GRL) have been shown to be related to diet in stickleback (Bentzen & McPhail, 1984; Schluter & McPhail, 1992; Robinson, 2000) and other fish species (Gibson, 1988; MacNeill & Brandt, 1990; Lundsgaard-Hansen *et al.*, 2013).

In addition, we measured the overall morphometric shape using fifteen landmarks that were placed on standardized photographs using the software TPSDIG2 (Rohlf, 2006; Fig. 2) and then used MORPHOJ (Klingenberg, 2011) to analyse the landmark coordinates. Here, we first regressed partial warp scores against standard length of the fish to correct for allometry, followed by a PCA based on a covariance matrix using Procrustes distances of the regression residuals. Because allometric effects of body size may be retained, we subsequently tested each PC axis for a statistical association with standard length using linear models.

Parallelism and nonparallelism of phenotypic differentiation

To estimate the relative degree of phenotypic differentiation among populations, we estimated P_{ST}, an analogue to Q_{ST} (Spitze, 1993) based on phenotypic measurements from wild individuals, using the approach of Kaeuffer *et al.* (2012). We use P_{ST} as a unitless and scale-free proportional measurement of pairwise difference and also to infer divergent selection on a trait by comparison with neutral genetic marker F_{ST} (Merilä & Crnokrak, 2001). As pointed out by several authors (Hendry, 2002; Edelaar & Björklund, 2011), P_{ST} should only be used for the latter in evolutionarily young and closely related populations assuming similar intrapopulation variation and mutation rates. With these caveats in mind, we nevertheless compare P_{ST} values with their respective F_{ST} to infer divergent selection only between parapatric populations. We calculated pairwise P_{ST} between populations using each linear trait and the number of gill rakers separately and based on the scores of the first PC for all linear traits combined or separated into feeding or defence traits. This was also performed using the scores of the first PC based on morphometric shape. For each P_{ST} value, we estimated the 95% confidence interval using a resampling approach with 1000 replicates. To further assess the directionality of the parapatric phenotypic divergence, we performed pairwise *t*-tests using the number of gill rakers as well as size-corrected trait values for linear measurements (statistics not shown).

To estimate the relative contributions of *habitat* (lake or stream), *system* (Bern, Biel, Constance, Geneva) and their interaction on divergence between lake and stream stickleback, we estimated the percentage of nonerror variance explained by each factor and their interaction based on their respective sums of squares using a sequential ANOVA model (Langerhans & DeWitt, 2004; Eroukhmanoff *et al.*, 2009). Here, the *habitat* term ought to reflect parallel parapatric divergent adaptation. The *system* term should reflect variation explained between parapatric lake and stream systems and thus likely reflect historical contingencies or environmental differences between lake and stream systems. Finally, the *system* × *habitat* interaction should account for the combined effects of system-related historical contingency and ecotypic differentiation (Langerhans & DeWitt, 2004; Eroukhmanoff *et al.*, 2009). We calculated these estimates for all linear traits, the number of gill rakers, the scores of the first PC for all linear traits combined or separated into feeding or defence traits and the scores of the first PC based on morphometric shape.

Testing for ecological speciation

A core prediction of ecological speciation theory is that adaptive phenotypic divergence between populations suppresses gene flow beyond what is explained by geographical distance, that is, isolation by adaptation (Nosil *et al.*, 2009; Shafer & Wolf, 2013). To test this, we used P_{ST} and the geographical distance between parapatric populations to predict F_{ST} either on their own or combined. Because the strength of divergent selection may differ among traits and functional trait categories, we estimated P_{ST} for each trait as well as for the leading PC axis combining all traits, defence-related traits, feeding-related traits and shape. We measured the pairwise geographical distance as the minimal waterway distance between sampling sites (estimated in GOOGLE EARTH 6.1; Google, Mountain View, CA, USA). Because the stream gradient between parapatric sites may be a better predictor for the potential of gene flow than geographical distance (Caldera & Bolnick, 2008), we additionally performed all analyses using the altitudinal difference between sites instead of geographical distance (Table 1). Divergence values from all parapatric comparisons were included in these models ($N = 9$). Because we had three different population contrasts (two stream populations and one lake population) in the Bern and Geneva lake–stream systems, and to account for potential effects of pseudo-replication, we also calculated the same linear models using each only one out of three population contrasts from these systems. This results in nine different possible combinations comprising five parapatric comparisons each. We then compared the R^2 values from these reduced models to the observed R^2 value of the model using all parapatric comparisons with a one-sample *t*-test. If the resampled R^2 values do not differ from the observed value, the repeated use of some populations in different population contrasts within the same system should not affect the overall conclusion (Table S2).

Comparative parapatric differentiation

A powerful way to infer the pervasiveness of habitat-dependent parallel divergence among Swiss stickleback is to compare the Swiss systems with other parapatric lake–stream systems elsewhere in the world. For this, we used published data from comparable systems in Canada (Kaeuffer *et al.*, 2012) and Ireland (Ravinet *et al.*, 2013). We also added published data from two Swiss systems, comprising additional parapatric contrasts from Lake Geneva and Constance (Berner *et al.*, 2010). We obtained the parapatric F_{ST} estimates for these population contrasts from the summary tables in the respective publications and the original morphological data from the Dryad Digital Repository (doi: 10.5061/dryad.1960, 10.5061/dryad.k987h, 10.1111/jeb.12049). In all cases, we applied the same size correction as to the Swiss populations studied here (see above) except to the number of gill rakers, which we did not transform. We then estimated phenotypic differentiation based on P_{ST} for morphometric shape, the length and number of gill rakers, the length of the first and second dorsal spine as well as the length of the pelvic spine. Because different landmarks were used among the different studies to assess morphometric body shape, the trait loadings of each PC analysis may differ. Consequently we did not assess directionality for morphometric body shape. All statistical analyses were performed in R 2.14 (R core development team 2012).

Stable isotopes

To test for differences in resource use among individuals inhabiting contrasting environments within lake systems, we used a subset of ten individuals from each population from all lake–stream systems (i.e. excluding the two Neuchatel stream sites) for stable isotope analyses of nitrogen (^{15}N) and carbon (^{13}C). To establish baseline SI signatures for ^{15}N and ^{13}C, we collected primary consumers for each site, sampling benthic invertebrates for streams and pelagic zooplankton for lakes at or close to the sampling site, depending on whether lake fish were sampled in the lake or in a nearby stream. Baseline samples were collected syntopically with the fish and during the same time of year under the same standardized conditions. We collected pelagic zooplankton from each lake over three 15-minute plankton tows with a 170-μm net. We then concentrated the zooplankton and stored it in 95% ethanol. Although not filtered to remove predatory species, because all pelagic zooplankton samples were treated

Table 2. Comparison of pairwise phenotypic (P_{ST}) and genetic (F_{ST}) differentiation between lake and stream ecotypes of stickleback from Switzerland, Ireland and Canada. P_{ST}s are based on the PC1 scores for geometric morphometric body shape, the number of gill rakers or size-corrected lengths of gill rakers, first (FSL), second (SSL) or pelvic spine (PSL). See main text for details.

Region	System	Habitat contrast	References	Body shape	No. Gill raker	Gill raker length	FSL	SSL	PSL	F_{ST}
Switzerland	Constance	Lake–Stream	This study	0.240	0.064	0.165	0.528	0.503	0.578	0.029
	Geneva	Lake–Stream	This study	0.051	0.054	0.295	0.509	0.500	0.636	0.059
		Lake–Mouth	This study	0.078	0	0.275	0.487	0.402	0.614	0.037
		Stream–Mouth	This study	0.219	0.045	0	0	0	0	0.029
	Bern	Lake–Stream	This study	0.043	0	0.005	0	0	0	0
		Lake–Mouth	This study	0.035	0	0	0.014	0.030	0.075	0.007
		Stream–Mouth	This study	0.144	0	0.008	0.029	0.128	0.123	0
	Biel	Lake–Stream	This study	0	0.093	0.174	0.370	0.366	0.429	0.009
	Neuchatel	Stream–Stream	This study	0.126	0.167	0	0.061	0.063	0.013	0.020
	Constance South	Lake–Stream	Berner *et al.* (2010)	0.331	0.097	0.486	–	–	–	0.110
	Constance West	Lake–Stream	Berner *et al.* (2010)	0.003	0	0.306	–	–	–	0.030
	Geneva	Lake–Stream	Berner *et al.* (2010)	0	0	0.021	–	–	–	0
Ireland (Lough Neagh)	Ballinderry	Lake–Stream	Ravinet *et al.* (2013)	0	0.094	0.078	0.053	0.080	0.175	0.040
	Blackwater	Lake–Stream	Ravinet *et al.* (2013)	0.024	0.023	0.085	0.001	0	0.004	0.003
	Crumlin	Lake–Stream	Ravinet *et al.* (2013)	0	0	0	0	0	0	0.021
	Glenavy	Lake–Stream	Ravinet *et al.* (2013)	0	0.613	0.355	0.036	0.054	0.190	0.062
	Lower Bann	Lake–Stream	Ravinet *et al.* (2013)	0.016	0.074	0.087	0.025	0.018	0.035	0.001
	Maine	Lake–Stream	Ravinet *et al.* (2013)	0.173	0.587	0.170	0.014	0.017	0.140	0.053
	Moyola	Lake–Stream	Ravinet *et al.* (2013)	0.210	0	0.217	0.150	0.191	0.164	0.065
	Six mile water	Lake–Stream	Ravinet *et al.* (2013)	0	0	0.130	0.049	0.068	0.119	0.047
	Upper Bann	Lake–Stream	Ravinet *et al.* (2013)	0.002	0	0.079	0.006	0.003	0	0.001
Canada, British Columbia	Beaver	Lake–Stream	Kaeuffer *et al.* (2012)	0.427	0.016	0.209	0.097	0.055	0.151	0.192
	Boot	Lake–Stream	Kaeuffer *et al.* (2012)	0.606	0.635	0.236	0.186	0.229	0.261	0.178
	Joe's	Lake–Stream	Berner *et al.* (2010)	0.281	0.418	0.450	–	–	–	0.120
	Misty	Lake–Stream	Kaeuffer *et al.* (2012)	0.779	0.298	0.011	0.075	0.173	0.047	0.121
	Pye	Lake–Stream	Kaeuffer *et al.* (2012)	0.694	0.652	0.279	0.357	0.328	0.400	0.069
	Robert's	Lake–Stream	Kaeuffer *et al.* (2012)	0.377	0.027	0.013	0.144	0.123	0.023	0.045
	Village Bay	Lake–Stream	Kaeuffer *et al.* (2012)	0.287	0.467	0.080	0	0.014	0	0.046

similarly, errors introduced in baseline values from unwanted species were likely small and applied evenly to all samples. In streams, we collected 5–10 gastropods (Lymnaeidae) and stored them in 95% ethanol. We prepared the fish tissue as described by Paterson *et al.* (2006), modified to also incorporate baseline samples. Briefly, we excised a 1.5 × 0.8 cm piece of muscle tissue from the right flank of each fish specimen. For each lake, we pooled zooplankton samples into a single sample and used them as whole body homogenates; we did the same with the soft body of gastropods after their shells were removed. We subsequently dried all samples in an oven at 75 °C for 48 h. We then placed the dried samples in clean solvent-rinsed glass mortar and pestle and pulverized them into a homogenous powder. For each sample, we placed 0.25–0.28 mg of the powder in a tin capsule (3.2 mm; Elemental Microanalysis, Okehampton, UK), folded it into a small cube and placed it into a standard 96-well sample plate. Samples were processed at the Environmental Isotope Laboratory (University of Waterloo, ON, Canada) using a Micromass Isochrom-EA continuous flow stable isotope ratio mass spectrometer. Resulting SI ratios for each sample were given as deviations from standard reference materials (Pee Bee belemnite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). For quality control and assurance, laboratory standards (uwEILAB, Waterloo, ON, Canada) were analysed every five samples and we included 13% of all samples as duplicates (including all baseline samples).

To compare the trophic position among populations within systems, we corrected the obtained $\delta^{15}N$ values using population-specific baseline values following Post (2002). Trophic position differences > 1 typically indicate substantially different trophic levels among populations assuming a trophic enrichment of 3.4% for $\delta^{15}N$ (Post, 2002). Once converted to trophic position, we tested whether or not absolute mean differences in trophic positions among parapatric populations were significantly < 1 using 10 000 Monte Carlo randomizations of individuals within each population.

For the $\delta^{13}C$ values, we applied a simple 2-source mixing model as demonstrated by McCutchan *et al.* (2003) to generate a proportion of pelagic and benthic/littoral carbon sources for each individual within systems. Here, we used the pelagic and benthic/littoral

baseline $\delta^{13}C$ from each system as the two input sources, applying a 1.3 ‰ trophic enrichment factor (see McCutchan *et al.*, 2003). Because of the nature of the two-source mixing model, especially when applying a trophic enrichment factor, it is not abnormal for carbon source proportions to sometimes be > 1 or < 0. This is an inherent problem of simple 2-source mixing models, which likely oversimplify or incompletely characterize carbon sources within such complex systems. However, the application of more complex models would require $\delta^{13}C$ values of more sources. With these caveats in mind, we nevertheless used this model to gauge the relative carbon sources among populations within systems. Finally, we compared the proportions of carbon sources using a Wilcoxon test between parapatric systems. Overall, our carbon data do not allow inferences of diet specialization because $\delta^{13}C$ signatures may also be reflective of populations feeding in different habitats. However, it does allow to estimate the respective parapatric habitat contrasts. All phenotypic, genetic and stable isotopic data are available in the Dryad Digital Repository.

Results

Genetic differentiation

Of the 18 microsatellite loci genotyped, one (*Stn209*) was monomorphic in all samples and we discarded it from further analyses. For the remaining loci, the number of alleles per locus varied from 3 to 17. Heterozygosity within population samples, averaged across all loci, varied from 0.470 to 0.625 (mean 0.550, ± 0.061 SD; Table 1). Global F_{ST}s, calculated separately for each marker, did not statistically differ between putatively QTL-linked and unlinked markers ($W = 22$, $P = 0.301$). Comparing the expected heterozygosities of Swiss invasive populations to those from 58 native freshwater populations from across Europe revealed a slight but significant reduction in heterozygosity among the invasive populations in Switzerland (Fig. S1; mean H_E Swiss populations = 0.542, mean H_E European populations = 0.598, $t_{1,58} = 2.2$, $P = 0.035$), suggesting that the recent invasion was associated with a slight loss of genetic variation.

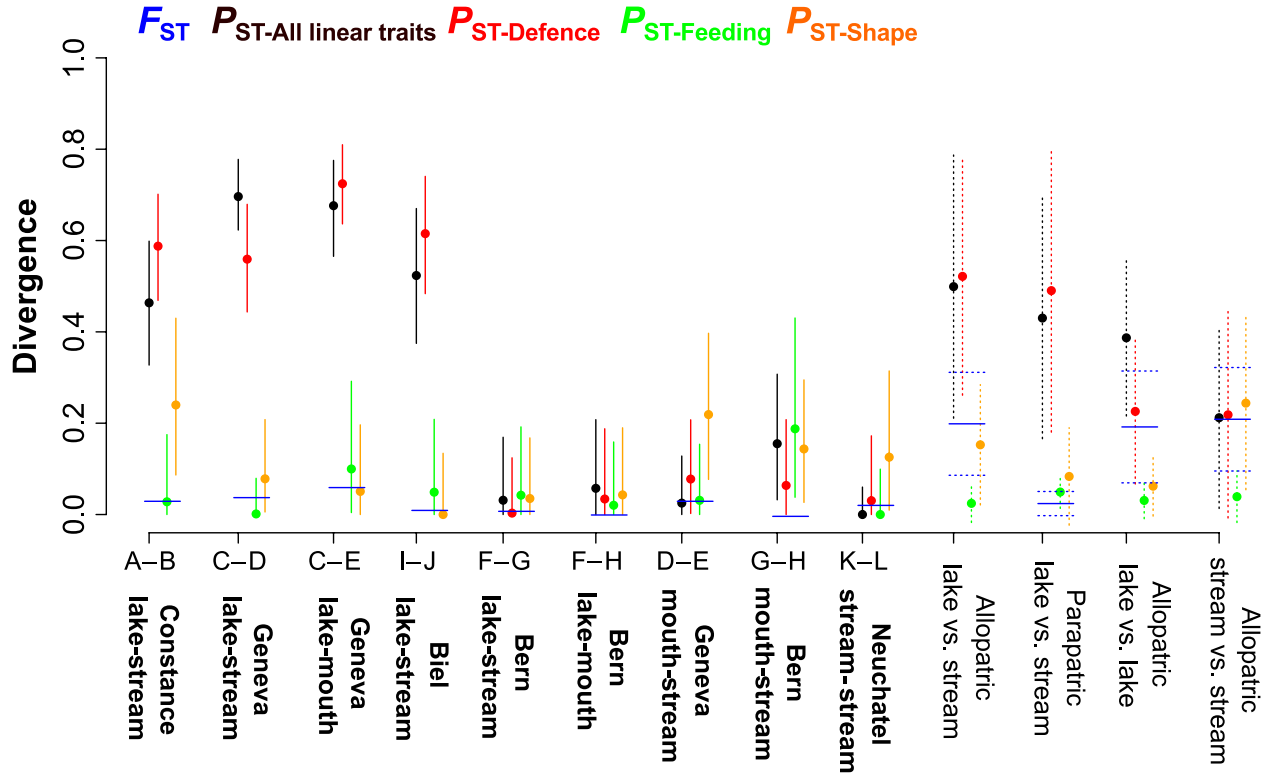

Fig. 3 Degree of parapatric genetic (F_{ST}) and phenotypic (P_{ST}) divergence as well as average degree of divergence among parapatric and allopatric lake–stream as well as allopatric lake–lake and stream–stream comparisons. P_{ST} was based on the individual scores of the first PC axis for either all linear traits combined or separately for defence and feeding traits (see Fig. 2) as well as the first PC for morphometric shape. Dots represent the average PST values with vertical bars representing their 95% confidence interval based on a resampling procedure with 1000 replicates (see main text for details). F_{ST} is given as solid blue horizontal lines. Dashed lines represent the standard deviation of allopatric comparison for both genetic and phenotypic data.

The degree of parapatric divergence did not statistically differ between unlinked and putatively QTL-linked markers (paired *t*-tests: *Stn26*: $t_{1,8} = 0.5$, $P = 0.631$; *Stn96*: $t_{1,8} = 0.8$, $P = 0.463$; *Stn130*: $t_{1,8} = 1.5$, $P = 0.165$; *Stn131*: $t_{1,8} = 1.2$, $P = 0.275$; *Stn152*: $t_{1,8} = 0.1$, $P = 0.902$; all QTL-linked markers combined: $t_{1,8} = 0.4$, $P = 0.669$) except for *Stn178* ($t_{1,8} = 3.1$, $P = 0.016$). However, in the latter case, F_{ST} values were significantly higher for unlinked markers (average $F_{ST} = 0.020$) than for *Stn178* ($F_{ST} = 0.001$), which may imply stabilizing selection on this marker. Certainly did this marker not drive parapatric genetic divergence. We consequently pooled all markers for all subsequent analyses. We found that the mean genetic differentiation was significantly lower among populations within a lake system (mean $F_{ST} = 0.038 \pm 0.051$) than among populations from different lake systems (mean $F_{ST} = 0.207 \pm 0.109$; $F_{1,63} = 23.16$, $P < 0.001$; Fig. 3). The AMOVA revealed that a much larger proportion of total genetic variance resided among lake systems (19.97%, d.f. = 4, $P < 0.001$) relative to between habitats within the lake systems (3.04%, d.f. = 7, $P < 0.001$). This provides a strong basis for our classification of lake–stream habitat pairs sampled within the same lake system as replicates of parapatric population divergence and populations from different lake systems as allopatric (Table S1). We consequently further refer to them as lake and stream populations. The neighbour-joining population tree further supports the classification into parapatric lake–stream populations pairs, showing that, with the exception of the geographically close Biel and Bern systems, samples from contrasting habitats in the same lake system are more closely related to one another than those from similar habitats in different lake systems (Fig. 4a). Populations from the Biel and Bern systems are all closely related such that sister pair relationships within these systems could not be resolved with confidence with our data. However, our data are still most consistent with parallel origins of lake and stream populations even between these geographically adjacent lake systems (Fig. 4a). The population tree shows two main clusters: one containing the two Constance populations and the other containing the three Geneva and the two Neuchatel populations (with 100% bootstrap support in each case). The populations from the Bern and Biel systems fall between these two main clusters, and the Neuchatel populations are intermediate too but closer to the populations from the Lake Geneva system, which reflects the different admixture proportions among three invasive lineages found in these systems (see Lucek *et al.*, 2010). STRUCTURE resolved parapatric populations from different habitats as distinct genetic clusters in both the Lake Constance and the Lake Geneva systems, whereas a single genetic cluster was observed in the Biel and Bern systems (Fig. 4b).

Parallelism and nonparallelism of phenotypic differentiation

Parapatric phenotypic differentiation (P_{ST}) differed among systems and traits (Fig. 5). Lake and stream populations differed the most in the Biel and Geneva system, where in each case, P_{ST} of nine linear traits exceeded the level of genetic differentiation (F_{ST}), followed by Constance with seven such strongly divergent traits. In the Bern system, P_{ST} of the lake population exceeded F_{ST} only for one trait (UJL), whereas for the comparisons that involved the population from the stream mouth, P_{ST} exceeded F_{ST} more often (three comparisons against the lake population and seven comparisons against the stream population). Similarly,

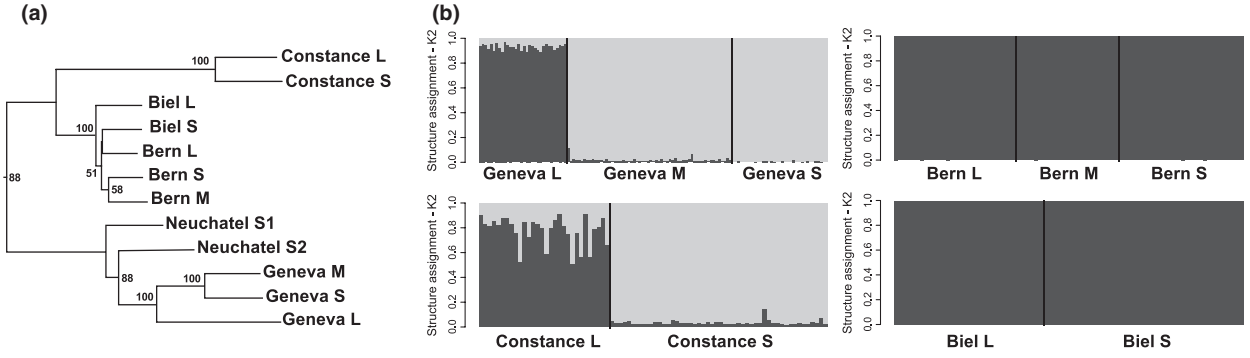

Fig. 4 Genetic differentiation among populations: (a) neighbour-joining tree (midpoint rooted) based on Nei's (1978) unbiased genetic distances among populations included in this study, calculated from allele frequencies at 17 microsatellite loci. Numbers beside nodes indicate percentage bootstrap support based on 1000 resampling replicates. Bootstrap values below 50% are not shown. (b) Genetic clustering inferred using STRUCTURE for each parapatric lake–stream system (Geneva, Constance, Biel, Bern) assuming two genetic clusters using sampling population as a prior. Because the best number of inferred clusters equalled one in Biel and Constance, they are represented as monomorphic clusters.

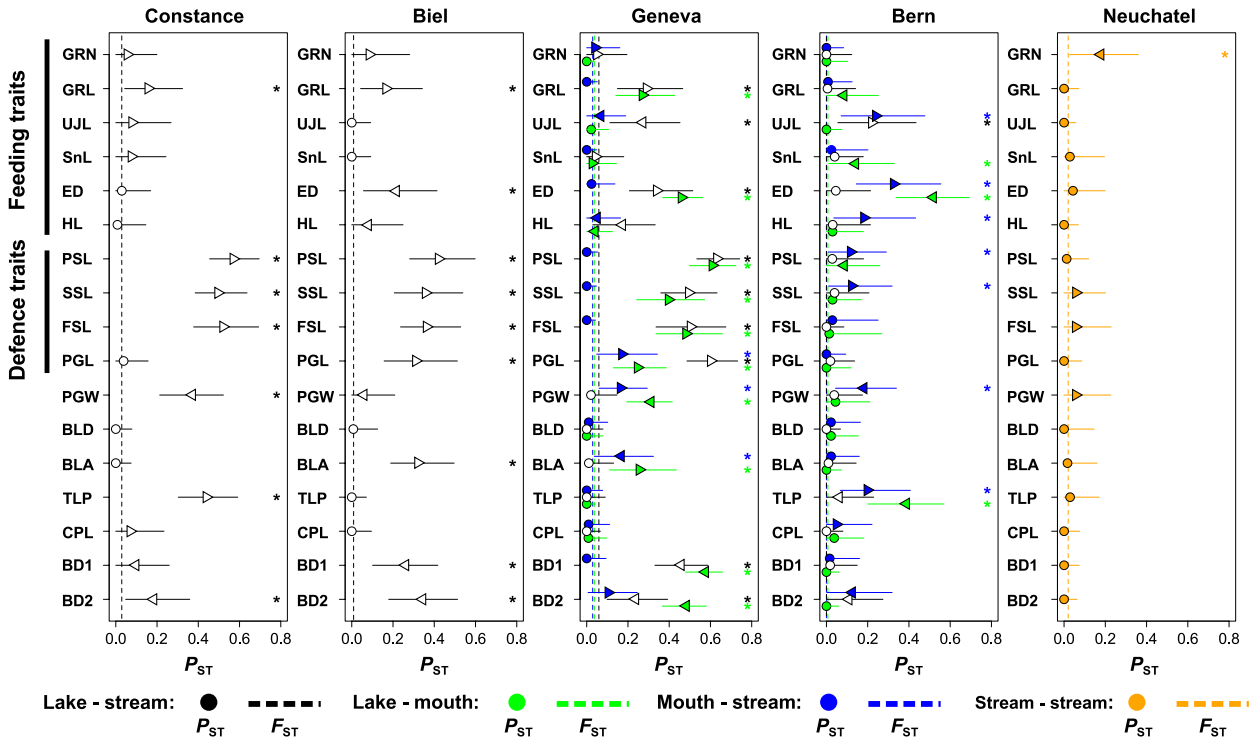

Fig. 5 Parapatric divergence ($P_{ST} \pm 95\%$ CI) for each linear trait (see Fig. 2). Circles depict cases where pairwise comparisons were not statistically significant ($P > 0.05$) based on a *t*-test, whereas triangles indicate significant pairwise comparisons ($P < 0.05$). The directionality of the triangle further indicates if the first-mentioned habitat is larger (pointing right) or smaller (pointing left) than second-mentioned habitat for each contrast. Parapatric F_{ST} for each comparison is plotted as dashed vertical line. Cases where $P_{ST} > F_{ST}$ are indicated with an asterisk.

the stream mouth population in the Geneva system differed from both the stream and the lake population, but was much more similar to the stream population than to the lake population (mouth–lake: ten comparisons; mouth–stream: three comparisons). Among the two stream populations that we compared in the Neuchatel system, only P_{ST} based on the number of gill rakers exceeded F_{ST}. Overall, antipredator-related defence traits differed most commonly between parapatric habitat contrasts, where lake fish showed elongated spines in comparison with stream and stream mouth populations. Divergence in feeding-related traits occurred frequently too, but only gill raker length showed parallel divergence in three lake–stream systems, where lake fish had longer gill rakers than stream and stream mouth fish (Fig. 5) Divergence occurred also in other feeding-related traits, but divergence was not repeated among systems. Finally, body depth showed parallel divergence in most lake–stream comparisons with stream fish being deeper-bodied than lake fish, which was equally true for the stream–mouth comparison in the Geneva system.

The first PC axis based either on all linear traits combined, on only defence traits or only feeding-related traits, explained 34.6%, 67.9% and 84.2% of the total variation, respectively. The first PC axis for morphometric shape accounted for 31.9% of the total shape variation. None of the PC axes for shape were associated with standard length (all $P > 0.99$). Parapatric P_{ST} based on PC scores using all traits or defence traits only exceeded F_{ST} to a similar degree in three lake–stream comparisons (Constance, Geneva and Biel) and the lake–mouth comparison in the Geneva system (Fig. 3). Parapatric P_{ST} of the stream–mouth comparison in the Bern system also exceeded F_{ST}, but to a lesser extent. Parapatric P_{ST} using only feeding-related traits exceeded F_{ST} only in the stream–mouth comparison within the Bern system. Differentiation in morphometric shape exceeded F_{ST} in the Constance lake–stream comparison and in both stream–mouth comparisons within the Bern and Geneva systems.

The magnitude of phenotypic differentiation between lake populations and between stream populations from different systems, that is, P_{ST} between allopatric ecotypes, was similarly high as that observed among parapatric ecotypes (PC1 all traits: $W = 33$, $P = 0.615$; PC1 defence traits: $W = 35$, $P = 0.727$; PC1 feeding traits: $W = 63$, $P = 0.071$; PC1 morphometric shape:

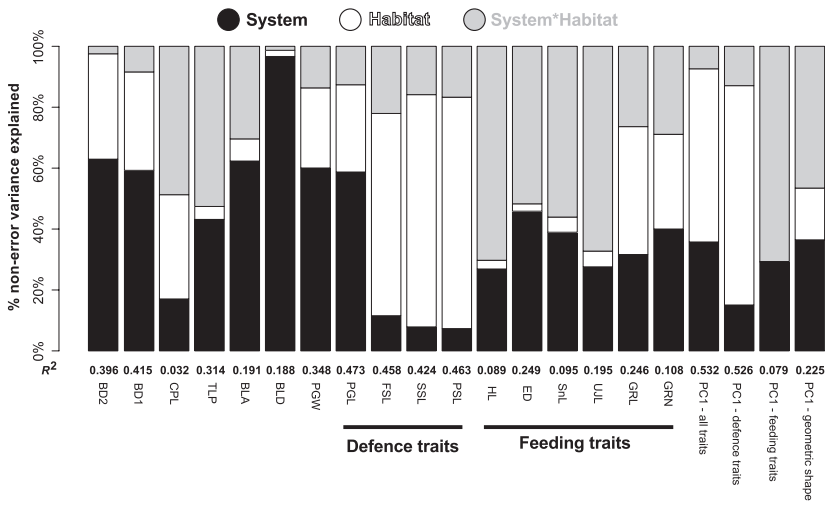

Fig. 6 Percentage of nonerror variation explained for the difference among parapatric lake–stream systems (Constance, Geneva, Bern, Biel), the difference between habitats (lake or stream) as well as their interaction for each linear morphometric trait. The R^2 values below each bar further indicate the overall amount of variation explained by each model.

$W = 27.5$, $P = 0.352$; Fig. 3). Although P_{ST}s derived from the PCAs combining either all traits, defence traits or feeding traits between allopatric populations from the same habitat were on average lower than for parapatric habitat contrasts (Fig. 3), they did not statistically differ between allopatric and parapatric comparisons (lake–lake vs. lake–stream: PC1 all traits: $W = 15$, $P = 0.610$; PC1 defence traits: $W = 18$, $P = 0.257$; PC1 feeding traits: $W = 17$, $P = 0.331$; PC1 morphometric shape: $W = 12$, $P = 0.999$; stream–stream vs. lake–stream: PC1 all traits: $W = 42$, $P = 0.152$; PC1 defence traits: $W = 44$, $P = 0.100$; PC1 feeding traits: $W = 38$, $P = 0.312$).

The trait-based ANOVA models all explained a significant amount of variation (average $R^2 = 0.288 \pm 0.160$ SD, Fig. 6; all $P < 0.001$, results not shown), where the lake system explained a significant ($P < 0.05$) amount of variation in all traits except HL and CPL (results not shown; average explained variation by *system*: 38.8% ± 22.3% SD). Differentiation between systems was highest for traits related to body shape or swimming behaviour, which was especially true for BLD (Fig. 6). *Habitat*, which is related to parallelism in parapatric lake–stream differentiation, explained on average a similar amount of the phenotypic variation (29.7% ± 26.5% SD; paired *t*-test for the percentage of variance explained by *system* and *habitat*: $t_{1,20} = 20$, $P = 0.344$). The habitat-related component was particularly large in spine lengths, gill raker length and gill raker number as well as body depth. Similarly, the scores of the leading axis of PCA based on either all linear traits or only defence traits showed a relatively high proportion of habitat-dependent variation. Finally, the *system* x *habitat* interaction explained on average 31.6% (± 23.2% SD) of the phenotypic variation, suggesting some system-specific component to parapatric lake–stream divergence especially for feeding-related traits and to a lesser extent for body shape.

Comparative analysis of lake–stream differentiation

The obtained values for P_{ST} from the Canadian parapatric lake–stream ecotypes differed from the values reported earlier of the same data set (Kaeuffer *et al.*, 2012; reanalysed in Ravinet *et al.*, 2013) for size-corrected shape and gill raker length but not for the number of gill rakers (Fig. S2). This may reflect differences due to the different size correction methods applied in each publication. The values reported here based on size-corrected data were closer to the ones reported by Kaeuffer *et al.* (2012) (shape: $R^2 = 0.415$; gill raker length: $R^2 = 0.431$) than Ravinet *et al.* (2013) (shape: $R^2 = 0.089$; gill raker length: $R^2 = 0.219$; Fig. S2) but that does not change any of the general patterns reported in these studies. Treating all data the same way, we can now compare the extent of parallel and nonparallel divergence among the different systems and studies. The comparative P_{ST} and F_{ST} values showed that whereas the largest differentiation for F_{ST} was observed in Canadian lake–stream systems (Canada vs. Europe: $t_{1,21} = 3.1$, $P = 0.015$), the degree of phenotypic differentiation can be as high in Europe as in Canada or higher (Fig. 7; Table 2). The differentiation of parapatric ecotypes in body shape was significantly higher in the Canadian systems ($t_{1,21} = 5.1$, $P = 0.001$). Similarly, gill raker number ($t_{1,21} = 2.2$, $P = 0.049$) showed an increased differentiation in the Canadian systems and also in two of nine comparisons from Lough Neagh (Ireland), compared with the Swiss and other Irish comparisons. However, with a single exception from Switzerland, the direction of divergence was consistent across all divergent ecotype pairs with lake populations having more gill rakers. Gill raker length was also very consistently divergent between lake and stream ecotypes with lake fish having significantly longer gill rakers in almost all cases. Interestingly, the magnitude of divergence in this trait was not

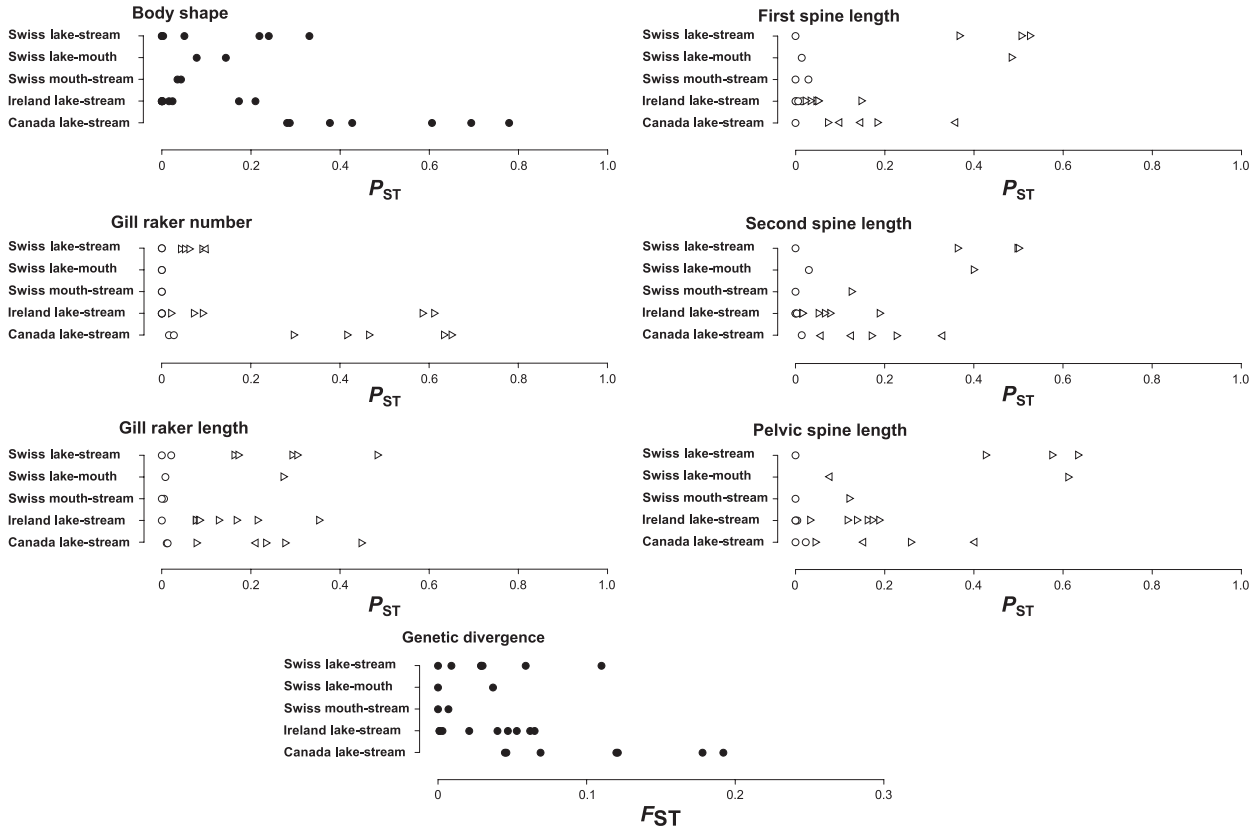

Fig. 7 Comparison of P_{ST} and F_{ST} values between parapatric stickleback ecotype pairs from Canada, Ireland and Switzerland. The directionality of differentiation for linear phenotypic measurements and for the number of gill rakers was statistically inferred using a *t*-test, where triangles indicate significant ($P < 0.05$) and open circles nonsignificant ($P > 0.05$) pairwise comparisons. For significant comparisons, the directionality of the triangle indicates if the first-mentioned habitat is larger (pointing right) or smaller (pointing left) than the second-mentioned habitat for each contrast. Filled circles depict the pairwise P_{ST} for geometric morphometric body shape and the pairwise genetic divergence based on F_{ST}.

different between Canadian and European systems ($t_{1,21} = 0.2$, $P = 0.811$). Finally, Swiss ecotypes exhibited the largest extent of phenotypic divergence in spine lengths. In all European systems with ecotypic differentiation, lake fish have longer spines than stream fish, albeit the difference is smaller in Ireland. The same divergence is not consistently observed in Canadian lake–stream comparisons, where lake fish can have either longer or shorter spines.

Consistent differentiation in trophic ecology

Although parapatric ecotypes from Switzerland showed differentiation in their trophic position in all instances, the mean differences were all significantly smaller than 1 ($P < 0.001$; Fig. 8a). This indicates that stickleback populations in all systems share a similar mean trophic position. The direction of divergence in trophic position between lake and stream stickleback varies among systems. The proportion of carbon obtained from a pelagic born source was also highly variable within systems (Fig. 8b). A consistent parallel pattern seen in all sampled lake–stream contrasts suggests that lake populations incorporate a significantly higher proportion of pelagic carbon in their diets than do the stream and stream mouth populations (Constance lake vs. stream: $W = 96$, $P < 0.001$; Geneva lake vs. mouth: $W = 100$, $P < 0.001$; Geneva lake vs. stream: $W = 100$, $P < 0.001$; Geneva mouth vs. stream: $W = 100$, $P < 0.001$; Bern lake vs. mouth: $W = 84$, $P = 0.009$; Bern lake vs. stream: $W = 100$, $P < 0.001$; Bern mouth vs. stream: $W = 86$, $P = 0.005$; Biel lake vs. stream: $W = 100$, $P < 0.001$). The stream mouth population from the Geneva system was more similar to the stream population from higher upstream, whereas the stream mouth population from the Bern system was on average intermediate to the lake and stream populations and showed a high variation among individuals.

Evidence for isolation by adaptation

F_{ST} was not significantly predicted by the waterway distance ($F_{1,7} = 3.9$, $P = 0.090$) nor by the differences in altitude ($F_{1,7} = 0.1$, $P = 0.740$). On the contrary, linear

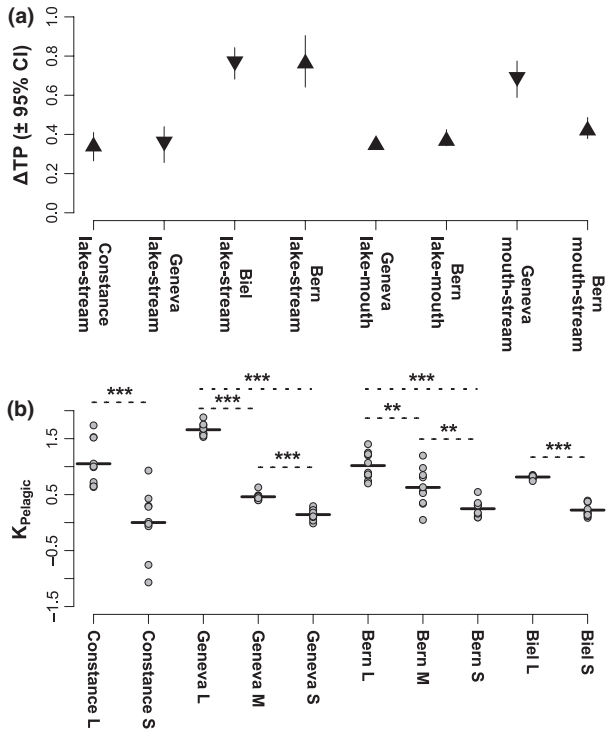

Fig. 8 (a) Mean trophic level differences among parapatric stickleback ecotypes in Switzerland. 95% confidence intervals calculated from 10 000 Monte Carlo randomizations. The directionality of the triangle further indicates if the first-mentioned habitat is larger (pointing up) or smaller (pointing down) than second-mentioned habitat for each contrast. All differences were found to be significantly smaller than 1 showing that ecotypes share a single trophic level ($P < 0.05$). (b) Proportion of individual carbon (collection mean indicated by black line) originating from pelagic sources determined using a simple 2-source mixing model. The statistical significance of pairwise differences between parapatric ecotypes are indicated based on Wilcoxon tests (**$P < 0.01$, ***$P < 0.001$). See main text for details.

models showed that P_{ST} in BD1 ($F_{1,7} = 5.8$, $P = 0.047$), FSL ($F_{1,7} = 6.0$, $P = 0.044$), PGL ($F_{1,7} = 9.2$, $P = 0.019$) and GRL ($F_{1,7} = 7.8$, $P = 0.030$) significantly predict F_{ST} (see Table S2 for details). Although waterway distance and the difference in altitude between our lake, stream and stream mouth populations were significantly correlated ($F_{1,7} = 9.3$, $P = 0.019$), none of the models using the altitudinal differences were significant (all $P > 0.100$, results not shown). Therefore, we report only results based on waterway distances. We found support for isolation by adaptation in the form of a significant effect of P_{ST} on F_{ST} when isolation by distance was controlled for in four traits (BD1: $F_{2,6} = 8.3$, $P = 0.018$, FSL: $F_{2,6} = 6.8$, $P = 0.029$, PSL: $F_{2,6} = 6.3$, $P = 0.034$, GRL: $F_{2,6} = 8.8$, $P = 0.016$) and in PC1 using all traits ($F_{2,6} = 5.6$, $P = 0.043$). For these models, adding P_{ST} to waterway distance for predicting F_{ST} led to a substantial increase in R^2 values (Table S2). Two of these results may have been affected by pseudoreplication, where the observed R^2 values were larger than the R^2 values from the resampled models (see Methods): BD1 ($t_{1,8} = 2.70$, $P = 0.027$) and GRL ($t_{1,8} = 2.45$, $P = 0.040$). Taken together, these results are consistent with predictions of isolation by adaptation and hence suggest the initiation of the process of ecological speciation (Nosil *et al.*, 2009; Nosil, 2012; Shafer & Wolf, 2013).

Discussion

Many unanswered questions remain regarding the relative importance of genetic, ecological and geographical constraints to adaptive evolutionary diversification of lineages, and often times empirical tests lag behind theory (Gavrilets & Losos, 2009; Nosil, 2012; Abbott *et al.*, 2013). Unresolved issues are related to the balance between adaptive divergence and gene flow and to the general relationship between these forces. Gene flow may often constrain adaptive divergence such that populations would be more divergent if gene flow was absent (Garant *et al.*, 2007; Räsänen & Hendry, 2008). Gene flow, however, can also promote adaptive divergence (Garant *et al.*, 2007; Räsänen & Hendry, 2008; Edelaar & Bolnick, 2012; Abbott *et al.*, 2013). Invasive species are useful models to address questions about the onset of adaptive diversification (Prentis *et al.*, 2008; Westley, 2011). Using the recent invasion of Swiss waterways by stickleback, where populations occupy a wide range of habitats and harbour much increased trait variation relative to individual source populations in their native range (Lucek *et al.*, 2010), we addressed some of these questions regarding the onset of diversification. We asked whether the wide habitat occupation and increased trait variation were associated with ecotypic differentiation between major habitats. We assessed whether or not the direction of differentiation was repeatable, whether it was predictable by the habitat contrast and whether it was constrained by gene flow. Hence, we tested for ecology-driven evolutionary differentiation within the invasive range, which may be considered the first phase in adaptive radiation (Schluter, 2000). Finally, we evaluated whether phenotypic divergence predicted genetic differentiation at neutral marker loci, which would indicate the initiation of the process of ecological speciation (Schluter, 2000; Nosil, 2012).

Replicated parapatric ecotypic differentiation among Swiss lake–stream systems

In stickleback, habitat-dependent phenotypic divergence between lake and stream populations has been shown to occur through adaptive phenotypic plasticity (e.g. Wund *et al.*, 2008; Leaver & Reimchen, 2012) as

well as through selection on standing genetic variation (Deagle *et al.*, 2012). The relative importance of each may depend on the investigated trait and population. Especially, antipredator-related traits often diverge between contrasting habitats as a consequence of divergent predation regimes (Reimchen, 1980, 1994; Marchinko, 2009). Similarly, adaptation to different feeding strategies is also thought to drive ecological divergence between benthic feeding stream populations and often more limnetic feeding lake populations (Berner *et al.*, 2008; Kaeuffer *et al.*, 2012). Body shape and especially body depth may diverge due to habitat-related differences in flow regimes and requirements for swimming behaviour (Bergstrom, 2002; Wark & Peichel, 2010; Hendry *et al.*, 2011). Many of the underlying traits that experience divergent selection have been shown to be heritable, including gill raker numbers (Hagen, 1973; Hermida *et al.*, 2002), spine length (Dingemanse *et al.*, 2009) and body depth for populations from Canada (Berner *et al.*, 2011). Feeding-related head shape on the other hand seems rather plastic in those populations (Wund *et al.*, 2008; Berner *et al.*, 2011). In Swiss stickleback, experimental work, focusing on feeding-related divergence, suggests a combination of both heritable and plastic components. In particular, feeding-related head shape is rather genetically determined and body depth is rather plastic (K. Lucek, A. Sivasundar & O. Seehausen, unpublished data).

Here, we investigated parapatric populations within five lake systems in Switzerland that differ from most of the studied lake–stream ecotype pairs from elsewhere in three key features: First, the time available for ecotypic divergence, with our lake–stream pairs ranging in age between < 90 and 140 years, whereas most other studies investigated much older lake–stream pairs (e.g. Berner *et al.*, 2009). Ecotype formation within freshwaters on a similarly recent contemporary timescale has only been investigated in two other cases: two other Swiss population contrasts in the Constance and Geneva system (~140 years; Berner *et al.*, 2010) and in California (< 50 years Hendry *et al.*, 2013). Second, the evolutionary history of Swiss populations, which derive most likely only from divergent freshwater lineages that independently colonized different European river systems post-glacially (Mäkinen & Merilä, 2008; Lucek *et al.*, 2010). This contrasts with the lake–stream systems that have been studied in Canada that evolved directly from marine ancestors, possibly through double invasion processes (Taylor & McPhail, 2000; Schluter & Conte, 2009; Jones *et al.*, 2012b). Hence, the observed divergence among Swiss systems evolved via selection on standing genetic variation from freshwater populations rather than from an ancestral marine population. Finally, the magnitude of the habitat contrasts, where most of our studied lakes are much larger and deeper, and in that sense, more marine-like, than formerly studied lakes (Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013).

Despite being relatively young, we observe significant genetic differentiation between parapatric lake, stream and stream mouth populations in the Constance, Geneva and the Biel system (Table S1) but not in the Bern system. In addition, the two stream populations from different tributaries of Lake Neuchatel are genetically differentiated too (Table S1). Parapatric ecotypes are genetically most closely related to each other within lake systems except perhaps among the closely related Bern and Biel systems (Fig. 4a). This suggests that adaptation to the distinct habitat contrasts studied here occurred in parallel in at least three instances, that is, the Constance, Geneva and Bern/Biel systems. We find that overall morphological divergence exceeds the expectations from neutral genetic differentiation in most parapatric contrasts between different habitats. This is also true specifically for antipredator-related morphology, gill raker lengths and body depth (Fig. 5). All of these traits are known to experience habitat-dependent divergent selection in Canada (Reimchen, 1994; Robinson, 2000; Wark & Peichel, 2010). This suggests that divergent selection between habitats has driven phenotypic divergence since the colonization of Swiss waterways. In contrast to the linear trait measurements, significant divergence in overall body shape occurs only in some comparisons (Fig. 3). Together, these results imply that – independent of the lake system – the two habitat types induce analogous divergent selection pressure, related to predation and feeding ecology, leading to similar and consistent ecotypic divergence among stickleback populations. This is especially remarkable given that some of our studied ecotype pairs (Constance vs. Geneva) represent the descendants of distantly related and phenotypically very different European lineages (Lucek *et al.*, 2010). Hence, the parallelism that we observed between these systems trumped historical contingency, making our results a clear example of independent parallel evolution.

In contrast to the observed habitat-dependent phenotypic divergence, the relative trophic position of parapatric ecotypes in the food web based on nitrogen isotopic ratios was similar, independent of habitat (i.e. less than $\pm$ 1 trophic units Post, 2002). This suggests conservatism of stickleback trophic position between different habitats (Fig. 8). However, the proportion of carbon emanating from a pelagic source may suggest a trophic differentiation within this trophic position in each lake–stream system. In all parapatric contrasts, lake populations showed a significantly higher mean proportion of carbon derived from pelagic sources than their associated stream or stream mouth populations. Such differences are consistent with individuals from the lake feeding more pelagically on zooplankton and using fewer littoral-benthic born dietary sources

relative to their stream and stream–mouth counterparts. Our findings are in line with studies on diversification within lakes along the benthic–limnetic axis (Snowberg & Bolnick, 2008; Matthews *et al.*, 2010). Yet, they differ from Kaeuffer *et al.* (2012), who report the opposite pattern for diversification along the lake–stream axis potentially as a result of different flow regimes among their studied streams. Stomach content data further support dietary differentiation between lake and stream stickleback in Switzerland (Gross & Anderson, 1984; Lucek *et al.*, 2012; Moser *et al.*, 2012).

Overall, we observed the largest phenotypic contrasts in the three systems where we sampled populations from very different habitats, namely little streams vs. the shores of the very large and deep lakes Constance, Geneva and Biel. Much smaller differences were observed between the smaller and shallow man-made Lake Wohlen and associated streams and between two streams in the Neuchatel system. The strongest genetic structure is also seen in two of the systems with the largest habitat contrasts, Constance and Geneva (Fig. 4b).

Parallelism and nonparallelism of parapatric divergence

Because the occurrence and extent of parapatric population divergence depends on the underlying environmental and selective gradients (Endler, 1977; Doebeli & Dieckmann, 2003), parallel evolutionary divergence is only expected when the selective regimes are very similar among systems (Kaeuffer *et al.*, 2012). Cases of parapatric lake–stream stickleback systems provide both evidence for parallelism and nonparallelism in the realized trait-specific divergence that occur both on smaller geographical scales as well as between continents (Hendry & Taylor, 2004; Berner, 2009; Berner *et al.*, 2010; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013). Cases of nonparallelism may arise through different selective regimes in similar habitats (Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013), genetic constraints (Berner *et al.*, 2010) or the evolutionary time for divergence (Berner *et al.*, 2010; Hendry *et al.*, 2013).

Overall, our results suggest strong parallelism among Swiss ecotype pairs in habitat-dependent differentiation for spine lengths and the PC axis combining antipredator-related traits (Fig. 6). This is remarkable as studies of similar ecotypes from elsewhere in the world did not find strong parallelisms for defence-related traits (Deagle *et al.*, 2012; Kaeuffer *et al.*, 2012; Ravinet *et al.*, 2013). This could imply that selective regimes among different Swiss waterways are more similar than those among waterways elsewhere. Similar selective regimes are also suggested by the parallelism in gill raker length and number, as well as body depth, but these are shared also with ecotype pairs from elsewhere (Kaeuffer *et al.*, 2012). On the other hand, especially morphometric shape and linear traits that are linked to body shape and swimming behaviour show a higher system-specific variation than, for example, spine lengths, which may point to lineage-specific historical contingencies. Finally, the system and habitat interaction that accounts for the combined effect of system-related historical contingency and parallel ecotypic divergence is highest for feeding-related traits.

Among the previous studies on lake–stream divergence in stickleback, the strongest parapatric divergence was observed in British Columbia (Canada) for morphometric shape, gill raker numbers, as well as, for genetic divergence (Berner *et al.*, 2010; Kaeuffer *et al.*, 2012). Less divergence was found in much younger ecotype pairs from Switzerland (Berner *et al.*, 2010). In the latter case, the authors suggested that time for divergence and genomic constraints might be responsible for the relatively minor phenotypic divergence. It is indeed possible that European populations are genomically constrained relative to Canadian populations because some of the genetic variation that is found in the Pacific lineage was lost upon colonization of the Atlantic, and it is this Atlantic marine lineage from which the European populations are derived (Jones *et al.*, 2012a). In accordance with these earlier findings, we find that phenotypic divergence in morphometric shape and gill raker number is significantly lower in European populations than among the Canadian systems (Fig. 7). In contrast with these earlier findings though, we find that parapatric divergence in gill raker lengths is quite similar on both continents, where Swiss systems can be as divergent as Canadian systems. Differences in genetic constraints affecting variation in gill raker length or in the ability to express phenotypic plasticity for this trait between stickleback from the Pacific coast of North America and the Atlantic-derived European populations may account for the observed difference. Alternatively, differences in the selective regimes between lake and stream contrasts in Canada and Europe could explain the observed pattern although this seems unlikely. Most importantly, we find the strongest phenotypic divergence for antipredator-related traits in Swiss systems, much stronger than that reported in either Canadian or Irish systems. Perhaps this is explained by the larger habitat contrasts in the Swiss systems, where our studied lakes Constance, Geneva and Biel are generally larger and deeper than the lakes studied in Canada. The predator-driven selective regimes in these lakes may resemble a marine-like environment, where increased spine lengths are favoured (Reimchen, 1994).

Evidence for ecological speciation

The causal relationship between adaptive divergence and limits to gene flow is difficult to establish (Garant

et al., 2007; Räsänen & Hendry, 2008; Shafer & Wolf, 2013). Positive correlations can be interpreted either as gene flow constraining adaptive divergence or *vice versa*. One way to test the role of adaptive divergence is to compare multiple pairs of populations that differ in their opportunities for gene flow (Nosil & Sandoval, 2008; Berner *et al.*, 2009; Stelkens & Seehausen, 2009; Moser *et al.*, 2012), as we have performed here. Even though the general relationship between gene flow and adaptive divergence may still be difficult to resolve unambiguously (Räsänen & Hendry, 2008), in the present case, gene flow does not appear to impose much constraint on adaptive divergence for the traits that show strong parallelism in parapatric divergence across lake–stream systems. Conversely, differentiation at microsatellite loci is explained by a combination of both geographical distance and phenotypic divergence (Table S2). The use of neutral genetic markers to infer ecological speciation has some potential caveats. First, neutral markers can be affected by random processes such as drift, leading to the detection of false positive cases for ecological speciation. This applies especially when gene flow is low and divergence among all populations is high in the absence of divergent selection (Thibert-Plante & Hendry, 2010). Secondly, differences at neutral genetic markers may not necessarily reflect gene flow if a system is not at equilibrium. On the one hand, founder effects may cause stronger genetic differentiation than expected at equilibrium. Therefore, if two populations originate from two independent colonization events, founder effects or pre-existing genetic differentiation between the source populations could result in an underestimation of gene flow (Labonne & Hendry, 2010). On the other hand, if a large population splits into two in the absence of founder effects, the level of genetic differentiation at neutral genetic markers may be lower than at equilibrium and hence overestimating gene flow (Hendry *et al.*, 2000).

Albeit founder events may account to some degree for the allopatric genetic divergence among our studied lake–stream systems, the observed parapatric genetic divergence within each system should not be affected, as they seem to each originate from a single founder event (Fig. 3). Similarly, initial founder events seem to play only a minor role, as the genetic variation was only slightly reduced in comparison with other European populations (Fig. S1). In addition, testing for isolation by adaptation, only the models for spine and gill raker length as well as body depth were significant, which are traits that are known to experience habitat-dependent divergent selection. Thus, it appears that adaptive divergence especially for antipredator-related traits and potentially gill raker length and body depth have lessened the homogenizing effects of gene flow by increasing the reproductive isolation between ecotypes. Restrictions to gene flow through divergent natural selection and phenotypic divergence over and above the limitations imposed by geographical distance is furthermore indicated because phenotypic divergence among parapatric lake–stream contrasts is no less than among allopatric lake–stream contrasts, despite much smaller F_{ST} (Fig. 3). This is a prediction of the early stages of ecological speciation and, when replicated many times within a lineage, marks the potential onset of adaptive radiation (Schluter, 2000; Nosil *et al.*, 2009; Nosil, 2012; Shafer & Wolf, 2013). Our study therefore adds to the rare – but growing – evidence for the rapid evolution of partial reproductive isolation (e.g. Hendry *et al.*, 2000; Rolshausen *et al.*, 2009; see Nosil, 2012 for a review).

Conclusions

Taken together, we show that the very recent invasion of Switzerland by three-spined stickleback is associated with the initiation of eco-morphological differentiation between populations inhabiting different major habitats, large lake and stream that may potentially lead to ecological speciation and adaptive radiation. We show that the phenotypic axes of divergence are parallel and predictable for some trait categories in replicate lake–stream systems that evolved independently after colonization by distinctly different lineages. Most notably, we find patterns consistent with the hypothesis that phenotypic divergence between parapatric ecotypes restricts gene flow, signalling the earliest steps towards adaptive ecological speciation. The general implications of our results are two-fold. First, they suggest that parapatric ecotype formation can occur relatively fast and along parallel phenotypic trajectories in independent cases with similar environmental contrasts. Secondly, phenotypic parallelism in habitat-dependent divergence is seen despite different evolutionary histories of the different populations, suggesting a strong and consistent habitat-dependent selective regime.

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References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N. *et al.* 2013. Hybridization and speciation. *J. Evol. Biol.* **26**: 229–246.

Bentzen, P. & McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. *Can. J. Zool.* **62**: 2280–2286.

Bergstrom, C.A. 2002. Fast-start swimming performance and reduction in lateral plate number in threespine stickleback. *Can. J. Zool.* **80**: 207–213.

Bernatchez, L., Renaut, S., Whiteley, A.R., Derome, N., Jeukens, J., Landry, L. *et al.* 2010. On the origin of species: insights from the ecological genomics of lake whitefish. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**: 1783–1800.

Berner, D. 2009. Correction of a bootstrap approach to testing for evolution along lines of least resistance. *J. Evol. Biol.* **22**: 2563–2565.

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., Roesti, M., Hendry, A.P. & Salzburger, W. 2010. Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Mol. Ecol.* **19**: 4963–4978.

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

Bertin, L. 1925. Recherches bionomiques, biométriques et systématiques sur les épinoches (*Gastérostéidés*). *Ann. Inst. Océanogr. Monaco.* **2**: 1–204.

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

Caldera, E.J. & Bolnick, D.I. 2008. Effects of colonization history and landscape structure on genetic variation within and among threespine stickleback (*Gasterosteus aculeatus*) populations in a single watershed. *Evol. Ecol. Res.* **10**: 575–598.

Coyne, J.A. & Orr, H.A. 2004. *Speciation*, 1st edn. Sinauer Associates, Sunderland.

Deagle, B.E., Jones, F.C., Chan, Y.F., Absher, D.M., Kingsley, D.M. & Reimchen, T.E. 2012. Population genomics of parallel phenotypic evolution in stickleback across stream-lake ecological transitions. *Proc. Biol. Sci.* **279**: 1277–1286.

Dieckmann, U., Metz, J., Doebeli, M. & Tautz, D. 2004. *Adaptive Speciation*, 1st edn. Cambridge University Press, Cambridge.

Dingemanse, N.J., Van der Plas, F., Wright, J., Réale, D., Schrama, M., Roff, D.A. *et al.* 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. *Proc. Biol. Sci.* **276**: 1285–1293.

Doebeli, M. & Dieckmann, U. 2003. Speciation along environmental gradients. *Nature* **421**: 259–264.

Edelaar, P. & Björklund, M. 2011. If F(ST) does not measure neutral genetic differentiation, then comparing it with Q(ST) is misleading. Or is it? *Mol. Ecol.* **20**: 1805–1812.

Edelaar, P. & Bolnick, D.I. 2012. Non-random gene flow: an underappreciated force in evolution and ecology. *Trends Ecol. Evol.* **27**: 659–665.

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

Elmer, K.R., Lehtonen, T.K., Kautt, A.F., Harrod, C. & Meyer, A. 2010. Rapid sympatric ecological differentiation of crater lake cichlid fishes within historic times. *BMC Biol.* **8**: 1–15.

Endler, J.A. 1977. *Geographic Variation, Speciation, and Clines*, 1st edn. Princeton University Press, Princeton, NJ.

Eroukhmanoff, F., Hargeby, A., Arnberg, N.N., Hellgren, O., Bensch, S. & Svensson, E.I. 2009. Parallelism and historical contingency during rapid ecotype divergence in an isopod. *J. Evol. Biol.* **22**: 1098–1110.

Falush, D., Stephens, M. & Pritchard, J. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol. Ecol. Notes* **7**: 574–578.

Fatio, V. 1882. *Faune des vertébrés de la Suisse*, 1st edn. H. Georg, Genève.

Felsenstein, J. 2012. *PHYLIP (Phylogeny Inference Package) version 3.69*. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, WA.

Garant, D., Forde, S.E. & Hendry, A.P. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. *Funct. Ecol.* **21**: 434–443.

Gavrilets, S. & Losos, J.B. 2009. Adaptive radiation: contrasting theory with data. *Science* **323**: 732–737.

Gibson, R.N. 1988. Development, morphometry and particle retention capability of the gill rakers in the herring, *Clupea harengus* L. *J. Fish Biol.* **32**: 949–962.

Gross, H.P. & Anderson, J.M. 1984. Geographic variation in the gillrakers and the diet of European threespine stickleback, *Gasterosteus aculeatus*. *Copeia* **1**: 87–97.

Hagen, D.W. 1973. Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculeatus*. *Heredity* **30**: 303–312.

Hagen, D.W. & Gilbertson, L. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution* **26**: 32–51.

Haldane, J.B.S. 1948. The theory of a cline. *J. Genet.* **48**: 277–284.

Heller, C. 1870. Die Fische Tirols und Vorarlbergs. *Z. Ferdinandeums Tirol.* **5**: 295–369.

Hendry, A.P. 2002. QST > = ≠ < FST? *Trends Ecol. Evol.* **17**: 502.

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., Wenburg, J.K., Bentzen, P., Volk, E.C. & Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**: 516–519.

Hendry, A.P., Day, T. & Taylor, E.B. 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., 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., Nosil, P. & Rieseberg, L.H. 2007. The speed of ecological speciation. *Funct. Ecol.* **21**: 455–464.

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

Hendry, A.P., Hendry, A.S. & Hendry, C.A. 2013. Hendry Vineyard stickleback: testing for contemporary lake-stream divergence. *Evol. Ecol. Res.* **15**: 1–32.

Hermida, M., Fernandez, C., Amaro, R. & San Miguel, E. 2002. Heritability and "evolvability" of meristic characters in a natural population of *Gasterosteus aculeatus*. *Can. J. Zool.* **80**: 532–541.

Hubisz, M.J., Falush, D., Stephens, M. & Pritchard, J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Resour.* **9**: 1322–1332.

Hudson, A.G., Vonlanthen, P. & Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *Proc. Biol. Sci.* **278**: 58–66.

Ingram, T. 2011. Speciation along a depth gradient in a marine adaptive radiation. *Proc. Biol. Sci.* **278**: 613–618.

Jones, F.C., Chan, Y.F., Schmutz, J., Grimwood, J., Brady, S.D., Southwick, A.M. *et al.* 2012a. A genome-wide SNP genotyping array reveals patterns of global and repeated species-pair divergence in sticklebacks. *Curr. Biol.* **22**: 83–90.

Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J. *et al.* 2012b. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**: 55–61.

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.

Klingenberg, C.P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11**: 353–357.

Kristjánsson, B.K., Skúlason, S. & Noakes, D. 2002. Morphological segregation of Icelandic threespine stickleback (*Gasterosteus aculeatus* L). *Biol. J. Linn. Soc.* **76**: 247–257.

Labonne, J. & Hendry, A.P. 2010. Natural and sexual selection giveth and taketh away reproductive barriers: models of population divergence in guppies. *Am. Nat.* **176**: 26–39.

Langerhans, R.B. & DeWitt, T.J. 2004. Shared and unique features of evolutionary diversification. *Am. Nat.* **164**: 335–349.

Leaver, S.D. & Reimchen, T.E. 2012. Abrupt changes in defence and trophic morphology of the giant threespine stickleback (*Gasterosteus* sp.) following colonization of a vacant habitat. *Biol. J. Linn. Soc.* **107**: 494–509.

Leimar, O., Doebeli, M. & Dieckmann, U. 2008. Evolution of phenotypic clusters through competition and local adaptation along an environmental gradient. *Evolution* **62**: 807–822.

Lucek, K., Roy, D., Bezault, E., Sivasundar, A. & Seehausen, O. 2010. Hybridization between distant lineages increases adaptive variation during a biological invasion: stickleback in Switzerland. *Mol. Ecol.* **19**: 3995–4011.

Lucek, K., Sivasundar, A. & Seehausen, O. 2012. Evidence of adaptive evolutionary divergence during biological invasion. *PLoS ONE* **7**: e49377.

Lundsgaard-Hansen, B., Matthews, B., Vonlanthen, P., Taverna, A. & Seehausen, O. 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus* spp.). *J. Evol. Biol.* **26**: 483–498.

Maan, M.E. & Seehausen, O. 2011. Ecology, sexual selection and speciation. *Ecol. Lett.* **14**: 591–602.

MacNeill, D.B. & Brandt, S.B. 1990. Ontogenetic shifts in gill-raker morphology and predicted prey capture efficiency of the alewife, *Alosa pseudoharengus*. *Copeia* **1**: 164–171.

Mäkinen, H.S. & Merilä, J. 2008. Mitochondrial DNA phylogeography of the three-spined stickleback (*Gasterosteus aculeatus*) in Europe-evidence for multiple glacial refugia. *Mol. Phylogenet. Evol.* **46**: 167–182.

Mäkinen, H.S., Cano, J.M. & Merilä, J. 2006. Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Mol. Ecol.* **15**: 1519–1534.

Marchinko, K.B. 2009. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. *Evolution* **63**: 127–138.

Matthews, B., Marchinko, K.B. & Bolnick, D.I. 2010. Specialization of trophic position and habitat use by sticklebacks in an adaptive radiation. *Ecology* **91**: 1025–1034.

Mayr, E. 1963. *Animal Species and Evolution*, 1st edn. Harvard University Press, Cambridge.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**: 378–390.

Meirmans, P.G. & Van Tienderen, P. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* **4**: 792–794.

Merilä, J. & Crnokrak, P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *J. Evol. Biol.* **14**: 892–903.

Mori, S. & Takamura, N. 2004. Changes in morphological characteristics of an introduced population of the threespine stickleback *Gasterosteus aculeatus* in Lake Towada, northern Japan. *Ichthyol. Res.* **51**: 295–300.

Moser, D., Roesti, M. & Berner, D. 2012. Repeated lake-stream divergence in stickleback life history within a central European lake basin. *PLoS ONE* **7**: e50620.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

Nosil, P. 2012. *Ecological Speciation*, 1st edn. Oxford University Press, Oxford.

Nosil, P. & Crespi, B.J. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in Timema cristinae walking-sticks. *Evolution* **58**: 102–112.

Nosil, P. & Sandoval, C.P. 2008. Ecological niche dimensionality and the evolutionary diversification of stick insects. *PLoS ONE* **3**: e1907.

Nosil, P., Harmon, L.J. & Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.* **24**: 145–156.

Paterson, G., Drouillard, K.G. & Haffner, G.D. 2006. Quantifying resource partitioning in centrarchids with stable isotope analysis. *Limnol. Oceanogr.* **51**: 1038–1044.

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

Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. & Moczek, A.P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* **25**: 459–467.

Phillips, B.L. & Shine, R. 2006. Allometry and selection in a novel predator-prey system: Australian snakes and the invading cane toad. *Oikos* **112**: 122–130.

Post, D.M. 2002. Using stable isotopes to estimate trophic position models methods and assumptions. *Ecology* **83**: 703–718.
Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. & Lowe, A.J. 2008. Adaptive evolution in invasive species. *Trends Plant Sci.* **13**: 288–294.
R Development Core Team 2012. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL www.r-project.org.
Räsänen, K. & Hendry, A.P. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecol. Lett.* **11**: 624–636.
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.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? *Can. J. Zool.* **58**: 1232–1244.
Reimchen, T.E. 1994. Predators and morphological evolution in threespine stickleback. In: *The Evolutionary Biology of the Threespine Stickleback* (M.A. Bell & S.A. Foster, eds), pp. 240–276. Oxford University Press, Oxford.
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.
Robinson, B. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour* **137**: 865–888.
Roesti, M., Hendry, A.P., Salzburger, W. & Berner, D. 2012. Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. *Mol. Ecol.* **21**: 2852–2862.
Rohlf, F.J. 2006. *TPSDIG2 version 2.10*. Distributed by the author. Department of Ecology and 937 Evolution, New York State University, Stony Brook, NY.
Rolshausen, G., Segelbacher, G., Hobson, K.A. & Schaefer, H.M. 2009. Contemporary evolution of reproductive isolation and phenotypic divergence in sympatry along a migratory divide. *Curr. Biol.* **19**: 2097–2101.
Rundle, H.D., Nagel, L., Wenrick Boughman, J. & Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**: 306–308.
Sandlund, O., Gunnarsson, K., Jónasson, P., Jonsson, B., Lindem, T., Magnússon, K. *et al.* 1992. The arctic charr *Salvelinus alpinus* in Thingvallavatn. *Oikos* **64**: 305–351.
Schluter, D. 2000. *The Ecology of Adaptive Radiation*, 2nd edn. Oxford University Press, Oxford.
Schluter, D. & Conte, G.L. 2009. Genetics and ecological speciation. *Proc. Natl. Acad. Sci. USA* **106**(Suppl 1): 9955–9962.
Schluter, D. & McPhail, J.D. 1992. Ecological character displacement and speciation in sticklebacks. *Am. Nat.* **140**: 85–108.
Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R. *et al.* 2008. Speciation through sensory drive in cichlid fish. *Nature* **455**: 620–626.
Shafer, A.B.A. & Wolf, J.B.W. 2013. Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecol. Lett.* **16**: 940–950.
Slatkin, M. 1973. Gene flow and selection in a cline. *Genetics* **75**: 733–756.
Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science* **236**: 787–792.
Snowberg, L.K. & Bolnick, D.I. 2008. Assortative mating by diet in a phenotypically unimodal but ecologically variable population of stickleback. *Am. Nat.* **172**: 733–739.
Spitze, K. 1993. Population structure in *Daphnia obtusa* – quantitative genetic and allozymic variation. *Genetics* **135**: 367–374.
Stelkens, R. & Seehausen, O. 2009. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution* **63**: 884–897.
Taylor, E.B. & McPhail, J.D. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proc. Biol. Sci.* **267**: 2375–2384.
Terai, Y., Seehausen, O., Sasaki, T., Takahashi, K., Mizoiri, S., Sugawara, T. *et al.* 2006. Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *PLoS Biol.* **4**: e433.
Thibert-Plante, X. & Hendry, A.P. 2010. When can ecological speciation be detected with neutral loci? *Mol. Ecol.* **19**: 2301–2314.
Wark, A.R. & Peichel, C.L. 2010. Lateral line diversity among ecologically divergent threespine stickleback populations. *J. Exp. Biol.* **213**: 108–117.
Westley, P.A.H. 2011. What invasive species reveal about the rate and form of contemporary phenotypic change in nature. *Am. Nat.* **177**: 496–509.
Wund, M.A., Baker, J.A., Clancy, B., Golub, J.L. & Foster, S.A. 2008. A test of the "Flexible stem" model of evolution: ancestral plasticity, genetic accommodation, and morphological divergence in the threespine stickleback radiation. *Am. Nat.* **172**: 449–462.
Yoder, J.B., Clancey, E., Des Roches, S., Eastman, J.M., Gentry, L., Godsoe, W. *et al.* 2010. Ecological opportunity and the origin of adaptive radiations. *J. Evol. Biol.* **23**: 1581–1596.

Supporting information

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

Figure S1 Expected heterozygosities (H_e) of the European freshwater populations of stickleback studied by Mäkinen *et al.*, 2006 and the Swiss populations in this study.

Figure S2 Pairwise P_{ST} values for either body shape, the number of gill raker or gill raker length between lake and stream habitats for six lakes from British Columbia, Canada.

Table S1 Table of pairwise F_{ST} among all population pairs.

Table S2 Summary table of general linear models describing the amount of variation (R^2) and the respective p value for each model.

Data S1 Methodological details and description of the microsatellite markers used in this study.

Data deposited at Dryad: doi:10.5061/dryad.0nh60

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