DISENTANGLING THE ROLE OF PHENOTYPIC PLASTICITY AND GENETIC DIVERGENCE IN CONTEMPORARY ECOTYPE FORMATION DURING A BIOLOGICAL INVASION

Kay Lucek,[1,2,3] Arjun Sivasundar,[1,2,4] and Ole Seehausen[1,2]

[1]Department of Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, CH-3012 Bern, Switzerland

[2]Department of Fish Ecology and Evolution, Center for Ecology, Evolution and Biogeochemistry, EAWAG Swiss Federal Institute of Aquatic Science and Technology, CH-6047 Kastanienbaum, Switzerland

   [3]E-mail: kay.lucek@eawag.ch

[4]National Centre for Biological Sciences, Tata Institute for Fundamental Research, Bellary Road, Bangalore 560065, India

The occurrence of contemporary ecotype formation through adaptive divergence of populations within the range of an invasive species typically requires standing genetic variation but can be facilitated by phenotypic plasticity. The relative contributions of both of these to adaptive trait differentiation have rarely been simultaneously quantified in recently diverging vertebrate populations. Here we study a case of intraspecific divergence into distinct lake and stream ecotypes of threespine stickleback that evolved in the past 140 years within the invasive range in Switzerland. Using a controlled laboratory experiment with full-sib crosses and treatments mimicking a key feature of ecotypic niche divergence, we test if the phenotypic divergence that we observe in the wild results from phenotypic plasticity or divergent genetic predisposition. Our experimental groups show qualitatively similar phenotypic divergence as those observed among wild adults. The relative contribution of plasticity and divergent genetic predisposition differs among the traits studied, with traits related to the biomechanics of feeding showing a stronger genetic predisposition, whereas traits related to locomotion are mainly plastic. These results implicate that phenotypic plasticity and standing genetic variation interacted during contemporary ecotype formation in this case.

KEY WORDS: Adaptive divergence, ecotype formation, heritability, invasion biology, plasticity.

Contemporary phenotypic evolution associated with adaptation to ecologically contrasting environments is a common phenomenon especially during biological invasions, that is, the establishment and spread of a species in a non-native environment. Examples derive from various taxa including plants (Bossdorf et al. 2005; Calsbeek et al. 2011; Matesanz et al. 2012; Sultan et al. 2013), invertebrates (Huey et al. 2000; Carroll et al. 2001; Lee et al. 2003), and vertebrates (Reznick and Endler 1982; Hendry et al. 2000; Koskinen et al. 2002). The evolution of ecologically and phenotypically differentiated populations occupying different environments within the invaded range has however less often

been described (e.g., Hendry et al. 2000; Carroll et al. 2001; Koskinen et al. 2002; Phillips and Shine 2006; Calsbeek et al. 2011; Matesanz et al. 2012). Although contemporary phenotypic evolution may represent a common feature in biological invasions (see Reznick and Ghalambor 2001; Carroll et al. 2007; Westley 2011), the respective roles of genetic determination, phenotypic plasticity, or their interplay in promoting or impeding such rapid adaptive responses are still debated.

Depending on the amount of gene flow between habitats with different requirements for adaptation, phenotypic divergence can evolve fast if standing genetic variation in relevant genes permits
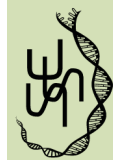
the emergence of beneficial phenotypes and their exposure to selection (Facon et al. 2006; Lee et al. 2007; Barrett and Schluter 2008). Sorting of the preexisting alleles can then lead rapidly to adaptive and heritable phenotypic differentiation between populations (Nosil 2012). In contrast, genetically depauperate populations would need time for advantageous genetic variation to arise, a process thought to be partly responsible for the so-called "lag phase" in biotic invasions (Sakai et al. 2001).

Beneficial phenotypes may also be expressed through phenotypic plasticity, where ancestral genotypes would be able to express different phenotypes in different environments (Price et al. 2003; Pfennig et al. 2010; Matesanz et al. 2012). Depending on the costs of plasticity and the stability of the selective regime, such divergent trait expression may itself become genetically fixed through phenotypic canalization and genetic assimilation or genetic accommodation (West-Eberhard 2003; Crispo 2008; Lande 2009; Thibert-Plante and Hendry 2011). Plasticity may however shield the genome from the effects of selection and hence prevent a genetic fixation (Carroll and Corneli 1999; Price et al. 2003; Ghalambor et al. 2007). The effects of plasticity on the strength of divergent selection further depends on its timing. If plasticity is expressed early in ontogeny before dispersal between contrasting habitats is possible, divergent selection can be strong because selection against immigrants can occur, whereas expression after dispersal may dissipate divergent selection (Thibert-Plante and Hendry 2011).

By raising wild populations with experimental treatments that mimic a key feature of habitat contrasts between ecotypes, we can experimentally test for the relative roles of plasticity and genetic determination in contemporary phenotypic evolution (Kawecki and Ebert 2004). Although this can only be achieved if divergence occurred recently, such as in recent biological invasions (Carroll et al. 2007; Lande 2009). The simultaneous assessment of the relative contributions of both plasticity and genetic differentiation that underlie adaptive trait divergence in such recently evolved systems are yet relatively rare (e.g., Weinig 2000; Carroll et al. 2001; Lee et al. 2003, 2011; Matesanz et al. 2012, see Carroll and Corneli 1999; Ghalambor et al. 2007 for a review), which is especially true for vertebrates (Robinson and Wilson 1996; Collyer et al. 2011). Disentangling the relative effects of genetics and plasticity is important because biological invasions that lead to the formation of distinct ecotypes can sometimes lead to ecologically differentiated species (Adams and Huntingford 2004) and even to adaptive radiations (Simpson 1953; Schluter 2000; Yoder et al. 2010).

The threespine stickleback (*Gasterosteus aculeatus* species complex) recurrently colonized freshwater environments from ancestral marine populations throughout its Holarctic distribution shortly after the last glaciation period ~15,000 years ago. In freshwater they repeatedly radiated into different habitat spe-

cialists, for example, in sympatry within a lake (McPhail 1984; Schluter and McPhail 1992) or between parapatric lake and stream environments (Reimchen et al. 1985; Hendry et al. 2002; Hendry and Taylor 2004; Berner et al. 2009; Kaeuffer et al. 2012; Ravinet et al. 2013), forming genetically distinct ecotypes and species. In many cases, the observed ecological differentiation among these taxa is manifested in functionally relevant phenotypic changes, for example, mouth shapes adapted to suction feeding on benthic invertebrates attached to the substrate in streams, compared to more ram feeding on zooplankton in lake habitats (Caldecutt and Adams 1998; McGee et al. 2013). Rearing experiments where stickleback were raised under a standardized food regime further suggest that phenotypic divergence along the lake–stream divergence axis can have a substantial genetic basis, at least in the only system where such studies have been performed (Misty Lake, Vancouver Island, Canada: Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008; Berner et al. 2011; see Table S1). Studies where populations were raised on divergent benthic and limnetic food regimes indicate that ecotypic differentiation can be partially attributed to adaptive phenotypic plasticity in a sympatric species pair (Day et al. 1994; Svanbäck and Schluter 2012) that is moreover present in the ancestral marine population (Wund et al. 2008). However, such studies have not been performed for parapatric lake and stream populations (Table S1).

Whereas many natural ecotype pairs began diverging shortly after the Pleistocene glaciation, sticklebacks arrived in the midlands of Switzerland only ~140 years ago (Lucek et al. 2010). Since then, they underwent a massive range and niche expansion, now occupying habitats as different as very large oligotrophic lakes, rivers, ponds, and small streams. Coinciding with this, repeated phenotypic divergence between physically connected lake and stream habitats occurred, forming lake–stream pairs that differ especially in feeding-related morphology and which are similar to those found within the native range (Lucek et al. 2013). One of the most strongly divergent of the known Swiss lake–stream population pairs occurs in the Lake Constance region, where stomach content data and stable isotopic signatures as well as morphological and life-history data suggest divergence into distinct ecotypes (Lucek et al. 2012, 2013; Moser et al. 2012). This ecotype pair originates from a single colonization event and is weakly genetically differentiated at neutral markers, but occasional gene flow may still occur (Lucek et al. 2010, 2013).

To test if the observed and potentially adaptive phenotypic differentiation of this ecotype pair results from environmentally induced plasticity or from divergent genetic predispositions, we used a controlled laboratory experiment (Day et al. 1994; Proulx and Magnan 2004; Wund et al. 2008). We raised full-sib F1 families from each ecotype under two food regimes, mimicking the main prey categories found in the wild (Lucek et al. 2012). We expected plasticity to importantly contribute to phenotypic

differentiation among ecotypes in our evolutionary young study system, because phenotypic plasticity has previously been found to contribute to ecotypic differentiation in stickleback populations that evolved since the end of the last glaciation period (e.g., Day et al. 1994; Wund et al. 2008). Thus, we predicted that feeding related plastic traits would differ according to the food treatment, whereas genetic predisposition should result in morphological differences between source populations independent of the food treatment. Both plastic and genetic effects may furthermore interact. Overall, we expected to observe phenotypic differences in feeding-related functionally relevant traits that would resemble the differences seen in the wild between the two ecotypes.

Material and Methods
FISH COLLECTION AND CROSSING
Adult individuals from Lake Constance, Switzerland (47°29′02″N, 9°33′35″E) and a connected (parapatric) stream site (47°19′33″N, 9°34′41″E) were sampled in May 2010. For each population, males with developed nuptial coloration and gravid females were randomly paired and placed in individual $60 \times 30 \times 40$ cm aquaria (one pair per tank). Each tank was equipped with sand substrate, natural nesting material, as well as a filtering and aerating system. Following a successful spawning event, both adult fish were removed and sacrificed with an overdose of anesthetic MS-222 and preserved in ethanol. A random population sample of wild adult fish was taken at the same time when collecting the parental fish ($N_{lake} = 96$, $N_{stream} = 49$) to obtain the phenotypic distributions from which the parents were drawn. The same was done in October 2010, at the end of the experiment, to obtain the phenotypic distributions of wild young of the year (YOY) individuals ($N_{lake} = 40$, $N_{stream} = 44$).

HUSBANDRY AND EXPERIMENTAL SETUP
Fertilized eggs were separately aerated in each tank. Eggs with fungal infection or dead embryos were removed daily. Two-thirds of the water in each tank was replaced every two days throughout the experiment. All hatched individuals were fed with *Artemia sp.* nauplii for the first five weeks after hatching. Between weeks 4 and 5, small nematodes (*Panagrellus* sp.) were also provided. After week 5, each full-sib family was split into two halves of 18–20 individuals each, experiencing from week 6 onwards either a "plankton"-type or a "benthos"-type food regime. For the plankton treatment, live zooplankton (mainly *Daphnia* sp. and limnetic copepods), collected with a 170 μm zooplankton net from Lake Lucerne, Switzerland, was provided as food every day. For the benthos treatment, live bloodworms (*Chironomidae* spp. larvae) were provided daily. These food items are similar to the main prey items eaten in the wild (Lucek et al. 2012). To furthermore re-

quire a more realistic benthic-type feeding behavior from the fish, bloodworms were introduced through a plastic tube separating them from the fish and allowing them to attach to the substrate. The plastic tube was then removed after five minutes, allowing the fish to feed by picking bloodworms out from the substrate. Fish were fed once per day till week 23 after hatching. After the experiment, all individuals were sacrificed with an overdose of anesthetic MS-222 and preserved in 95% ethanol. To highlight bony structures, all individuals were stained using a protocol from Peichel et al. (2001), followed by a bleaching step with a solution of 0.6% KOH and 1.2% H_2O_2.

MORPHOLOGICAL ANALYSIS
To quantify relevant phenotypic differentiation among groups, a set of morphological traits that are known to be often divergent among stickleback ecotypes was measured (Day et al. 1994; Berner et al. 2008): standard length, body depth, head length, head depth, eye diameter, upper and lower jaw length, snout length, and gape width. Standardized pictures were obtained from the left side of each stained fish with a flat-bed scanner on which all linear measurements were then taken using IMAGEJ 1.43u (Abràmoff et al. 2004), except for gape width. The latter was measured as the ventral distance between the posterior-most points of the premaxillary bones of each side to the nearest 0.01 mm using a digital caliper. In addition, the number of gill rakers, the gill arch length, and the length of the second gill raker, as counted from the joint of the dorsal arch bone and measured from its tip to the insertion on the gill arch, were determined using a dissection microscope with a micrometer attached. Because all measurements except gill raker numbers were significantly correlated with size (results not shown), a size correction was applied by taking the residuals of a regression of each untransformed linear trait against standard length. This was performed separately for the experimental individuals, wild-caught adults, and YOY using each a single within-group regression to account for allometric differences between these groups.

Overall multivariate differentiation among experimental fish was tested in three ways: first a linear discriminant (LD) analysis was performed using all linear traits with food treatment and source population separately as grouping variables to identify trait contributions associated with either response variable. The classification success, which is defined as the average probability among individuals for each group to be assigned to their own group, was then extracted from the LD model. In addition, the degree of differentiation between groups was estimated as their pairwise Mahalanobis distances. Second, an analysis of multivariate variance (MANOVA) was performed with family as a random factor to test for an overall statistical phenotypic differentiation between either food treatment or source population. Third, all linear traits were summarized using a principal component (PC)

analysis, retaining the scores for each individual for the three leading PC axes.

Each trait and PC axis was analyzed using a mixed linear model including *food treatment* (plankton or benthos) and *source population* (lake or stream) as explanatory variables and family as a random factor. The significance levels of the explanatory variables were assessed using a backward elimination procedure based on type II *F*-tests (see Lemoine et al. 2012 for details). The effect size of *food treatment*, *source population*, and the *food treatment × source population* interaction was further estimated using Cohen's *D* to quantify the relative contributions of plasticity and genetic predisposition.

Differentiation between habitats was similarly tested for the wild-caught populations separately for adults and YOY using an LD analysis and a MANOVA. Likewise, the classification success and the degree of differentiation measured as pairwise Mahalanobis distances were calculated. In addition, each measured morphological trait was separately compared between wild-caught populations using *t*-tests.

SHAPE ANALYSIS

Geometric morphometrics was used to capture shape variation in wild-caught and experimental fish. Nineteen landmarks were selected that cover the overall body shape with an emphasis on head shape and traits related to functional morphology of the feeding apparatus (Anker 1974; Walker 1997; Caldecutt and Adams 1998; McGee et al. 2013; Table S2). All landmarks were placed on dyed bone structures. Landmarks were set using TPSDIG2 (Rohlf 2006), with individuals in random order. Procrustes fits were performed on the obtained datasets separately for wild adults, wild YOY, and experimental fish in MORPHOJ 1.03b (Klingenberg 2011). Procrustes coordinates were size corrected by a regression against standard length retaining the residuals. A canonical variate (CV) analysis on these residuals was performed, based on pooled within group covariances to identify the multivariate axis that explains most variation between groups. Groups represented either source population (lake and stream) for both experimental and wild-caught fish or food treatment for experimental fish only. For each analysis, the classification success for each group was extracted from MORPHOJ (Klingenberg 2011). Furthermore the degree of differentiation among groups was estimated as pairwise Mahalanobis distances, whose significances were estimated using a bootstrap approach with 10,000 replicates implemented in MORPHOJ. In addition, a PC analysis was conducted retaining the scores of the three leading PC axes, where significances among groups were similarly calculated as for the linear measurements. To further illustrate the phenotypic changes associated with differences in CV scores, deformation wire frame graphs were 2.5 times exaggerated (Wund et al. 2008).

Trait loadings along each CV axis for shape data and along each LD axis for linear morphology were standardized for each axis separately by dividing the absolute trait loadings with the highest observed loading on each axis. To compare the observed differentiation along the LD or CV axes for experimental individuals in relation to their wild-type counterparts, the latter were furthermore projected into the morphospace of the experimental individuals using the package MASS (Venables and Ripley 2002) in R 2.15.1 (R Core Team 2012). In short, this projection approach uses the loading vectors of each LD or CV that separate either source populations or treatments for experimental individuals and calculates the residuals for each projected individual along each given axis. These projected scores can subsequently be analyzed. Similarly both the experimental individuals and wild-caught YOY were projected onto the multivariate axis that separates the wild-caught adult populations. Finally, the residuals of each LD, PC, and CV analysis were subsequently regressed against standard length, to test if the multivariate differentiation was driven by allometric information that might have been retained after the size correction. Similarly, MANOVAs were performed for linear measurements using size as a factor.

Lastly, to test if the parental individuals used to breed the experimental crosses represented a random sample of the phenotypic variation in the wild, they were projected onto the LD axis separating the larger sampling populations of wild-caught fish for liner measurements, and onto the respective CV axis for body shape. The obtained individual scores were then statistically compared between our breeders and the larger sample of wild-caught individuals (excluding the breeders) separately for the lake and stream population using *t*-tests.

Results

In total, 441 individuals were alive at the end of the experiment. Overall mortality was 13.9% ± 3.2 SE, and did not statistically differ between *food treatment* ($F_{1,22} = 0.04$, $P = 0.849$) or *source population* ($F_{1,22} = 2.54$, $P = 0.126$) with a nonsignificant interaction ($F_{1,22} = 3.46$, $P = 0.076$) between them. Fish tended to have higher mortality in their native treatment, but none of the pairwise comparisons were significant (all $P > 0.100$). Individuals in the plankton treatment were slightly but significantly larger than in the benthos treatment at the end of the experiment ($F_{1,426} = 3.91$, $P = 0.049$), but size did not differ between source populations ($F_{1,11} = 1.31$, $P = 0.278$) with a nonsignificant interaction ($F_{1,426} = 0.39$, $P = 0.531$).

LINEAR MORPHOLOGY—WILD FISH

Using size-corrected linear morphology, wild-caught adult fish differed between the stream and lake environment (MANOVA:

$F_{1,139} = 4.07$, $P < 0.001$, Mahalanobis distance: 1.167), which was similarly recovered with the LD analysis (Figs. 1, S1, Table S3). The classification success differed between the lake (lake fish assigned to the lake population: 70.8%) and the stream population (stream fish assigned to the stream population: 48.4%). In contrast, YOY individuals were not significantly differentiated in the overall multivariate analysis (MANOVA: $F_{1,82} = 1.01$, $P = 0.734$, Mahalanobis distance: 0.734). This was likewise reflected with the LD analysis, where the classification success was similar for the lake (lake fish assigned to the lake population: 48.5%) and the stream population (stream fish assigned to the stream population: 51.1%). However, YOY showed a significant differentiation in several traits between both environments (Table S4).

Wild adult lake fish had shallower bodies ($t_{1,139} = -3.98$, $P < 0.001$) and deeper heads ($t_{1,139} = 3.03$, $P < 0.003$) and were significantly larger than wild adult stream fish ($t_{1,139} = 9.73$, $P < 0.001$; Table S4). Gill raker number was not different ($t_{1,139} = -0.70$, $P = 0.487$) but gill rakers were significantly longer in the lake population ($t_{1,139} = 3.98$, $P < 0.001$). Wild-caught YOY from the lake were significantly smaller in size than YOY stream fish ($t_{1,82} = -12.06$, $P < 0.001$). Wild-caught stream YOY had significantly larger heads ($t_{1,82} = -5.58$, $P < 0.001$), eyes ($t_{1,82} = -8.15$, $P < 0.001$), lower jaws ($t_{1,82} = -4.54$, $P < 0.001$), longer gill rakers ($t_{1,82} = -3.29$, $P < 0.001$), and wider gapes ($t_{1,82} = -8.72$, $P < 0.001$; Table S4).

LINEAR MORPHOLOGY—EXPERIMENTAL FULL-SIB F1 FISH

For the experimental fish, the three leading PC axes captured 75.2% of the total variation (48.9%, 16.0%, 10.3% on PC axes 1–3, respectively) with PC1 being significantly associated with food treatment (Fig. 2, Table S5). PC2 and PC3 showed a significant *food treatment × source population* interaction and a significant *food treatment* factor, indicating different multivariate reaction norms of source populations (Fig. S2). Effects of food treatment and source population differed among traits when each trait was separately analyzed (Fig. 2, Table S5). Here, only gape width showed a significant interaction, indicating different reaction norms between source populations, with fish raised in their native like environment having wider gapes than in non-native like environment, where furthermore lake fish had wider gapes than stream fish in both food treatments (Fig. S2). Significance levels were consistent with effect sizes; most linear traits had a high treatment induced component, with the interaction showing the second strongest effect in most cases (Fig. 2). Individuals from the benthos treatment had shorter heads with smaller eyes and deeper bodies (Fig. S2). Some feeding-related traits (gill raker length, gill arch length, lower jaw length) showed no significant difference in any comparisons (Fig. 2, Table S5).

The multivariate analyses based on the linear measurements showed a significant separation between treatments (MANOVA: $F_{1,427} = 31.5$, $P < 0.001$, Mahalanobis distance: 1.630) and source populations (MANOVA: $F_{1,427} = 18.6$, $P < 0.001$, Mahalanobis distance: 1.312). This was similarly true for both LD analyses (Fig. 1A), where gape width contributed highly on both axes (Fig. S3, Table S3). The classification success was comparable among treatments (benthic-fed fish assigned to the benthos treatment: 69.7%; limnetic-fed fish assigned to the plankton treatment: 71.6%) and among source populations (lake fish assigned to the lake population: 64.7%; stream fish assigned to the stream population: 65.3%). When wild-type individuals were projected into the morphospace of experimental individuals, YOY clustered closely together and were intermediate to the experimental individuals along both LD axes (Fig. 1A). Wild-caught adult individuals on the other hand clustered closely to their matching experimental counterpart. This was especially true for wild stream adults, resembling benthic-fed experimental stream fish (Fig. 1A). However, when wild YOY and the experimental individuals were projected on the axis that separates wild-caught adult populations (Fig. 3), the experimental individuals segregate toward their matching ecotype.

SHAPE ANALYSIS—WILD FISH

Wild-caught adults but not YOY, differed significantly in their multivariate shape between source populations with a similar decreased differentiation between source populations among YOY as observed with linear morphology (adults: Mahalanobis distance: 1.660, $P < 0.001$; YOY: Mahalanobis distance: 0.960, $P = 0.995$). This was similarly reflected by the classification success, which was higher for adults (average probability for lake fish being assigned to the lake population: 82.9%; stream fish to the stream population: 84.9%) than for YOY (lake fish to the lake population: 60.0%, stream fish to the stream population: 68.2%). Decreased differentiation among YOY was furthermore observed with the CV analysis (Fig. S1). Yet, both adult and YOY stream fish showed deeper bodies and smaller eyes (Fig. 1). Head shapes differed among the wild-caught adults, where lake fish had more elongated and deeper heads and longer jaws. In YOY, this was inversed with deeper heads in stream fish. Landmarks accounting for most of the phenotypic variation between wild populations for both adults and YOY were concentrated on the head (Table S6). These traits are furthermore mechanically important for the relative forces applied during feeding (Caldecutt and Adams 1998; McGee et al. 2013).

SHAPE ANALYSIS—EXPERIMENTAL FISH

The three leading PC axes on shape for experimental individuals captured 57.0% of the total variation (29.3%, 17.1%, 10.6% on PC axis 1–3, respectively). The shape changes captured by these
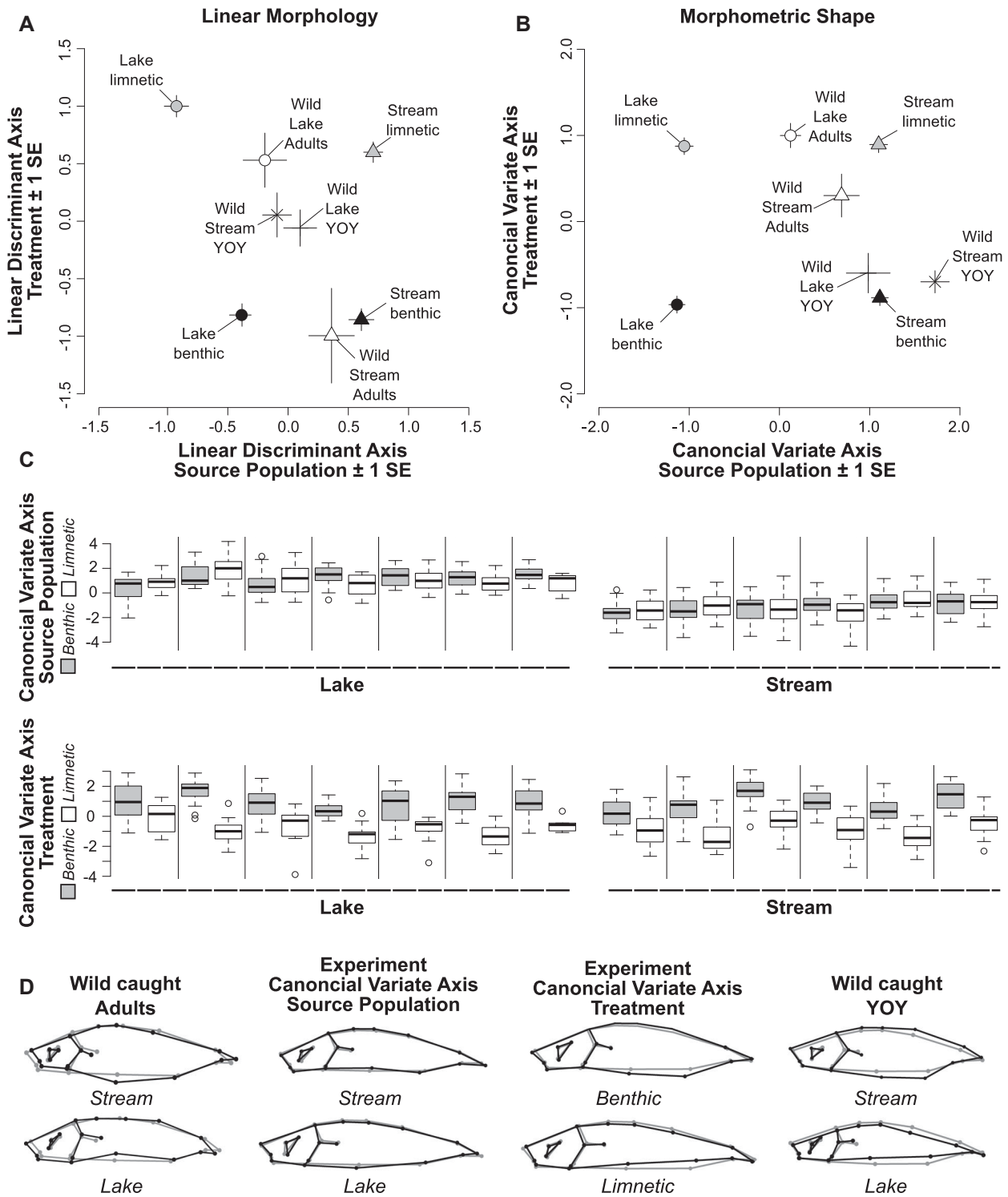
Figure 1. Summary of the phenotypic changes observed for both linear morphology and morphometric shape. (A) Average linear discriminant loadings (± 1 SE) on the axes separating either source population (lake, stream) or treatment (limnetic plankton, benthos). In addition, both wild-caught young of the year (YOY) and adult individuals were projected into the morphospace of the experimental individuals (see main text for details). (B) Average canonical variate loadings on the leading axes for source and treatment (± 1 SE) for all experimental fish with wild-caught individuals being projected into the morphospace of the experimental individuals. (C) Canonical variate scores separated for each family and treatment (gray—benthic or white—limnetic zooplankton) of the leading axis for morphometric shape data using either only source population (lake or stream; top) or treatment (benthic or limnetic zooplankton; bottom) as grouping variable. (D) Morphometric shape differences along the leading canonical variate axis for wild-caught adults, YOY, and experimental individuals. Deformations (black) are 2.5× exaggerated to visualize the differences from the consensus (gray).
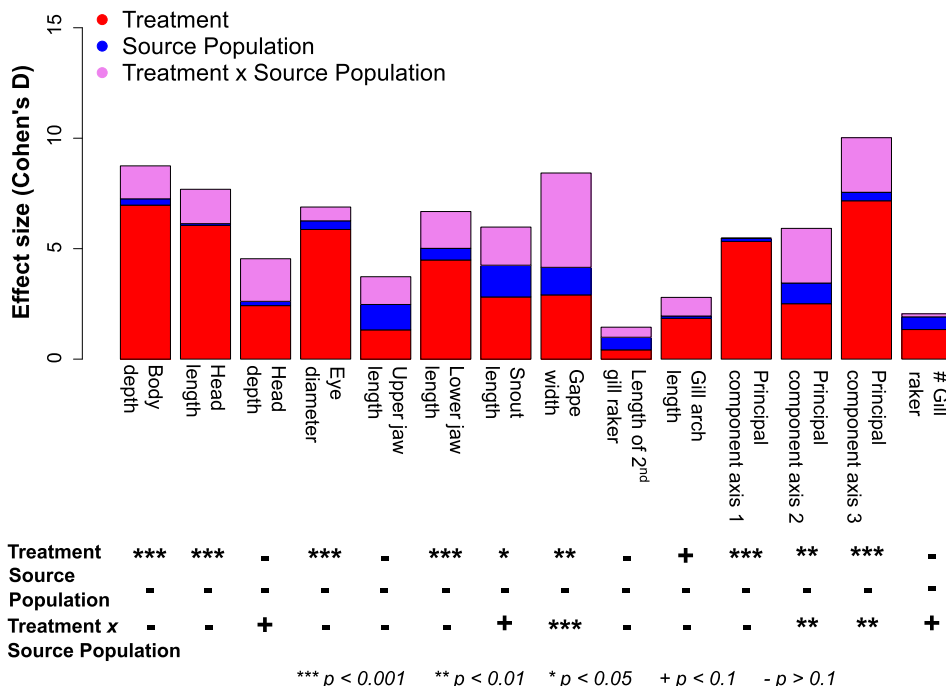
Figure 2. Effect sizes (Cohen's *D*) for treatment, source, and their interaction with the corresponding *P*-values are given for all linear size-corrected morphological traits separately, and for these traits combined using a principal component analysis (principal component axes 1–3 indicated). Results for the number of gill raker are shown separately. The significance levels are based on mixed linear models with source population and treatment as response variable and family as random effect using a backward elimination procedure and a type II *F*-tests (see Fig. S2 and Table S5 for details).

PC axes were however mainly related to changes in the bending of a specimen and the vertical position of the tail, where only PC3 showed some changes in body depth (Fig. S4). None of these axes showed a significant contribution of either *source, treatment*, or their interaction except for PC3, where the best statistical model showed a significant treatment effect ($F_{1,426} = 4.0$, $P = 0.047$; Table S5). The CV axes for experimental fish on the other hand significantly separated both source populations (Mahalanobis distance: 2.201, $P < 0.001$) and food treatments (Mahalanobis distance: 1.812, $P < 0.001$; Figs. 1, 3). The relative classification success was comparable between the CV axes separating *treatment* (benthic-fed fish assigned to the benthos treatment: 80.9%; limnetic-fed fish assigned to the plankton treatment: 83.1%) and *source* (lake fish assigned to the lake population: 86.4%; stream fish assigned to the stream population: 84.9%). This differentiation was remarkably consistent among all 13 families within each analysis (Fig. 1C). Traits that explained most variation on the CV axis, which separated experimental individuals according to their source population, involved changes along the anteroposterior axis, especially head shape: experimental individuals originating from the lake had a more terminal mouth with the maxilla dorso-caudally shifted and a shorter head (Table S6, Figs. 1B, 3). Experimental stream fish showed a reduced orbit size, being linked to eye size with an increased suspensorium size (suspending the
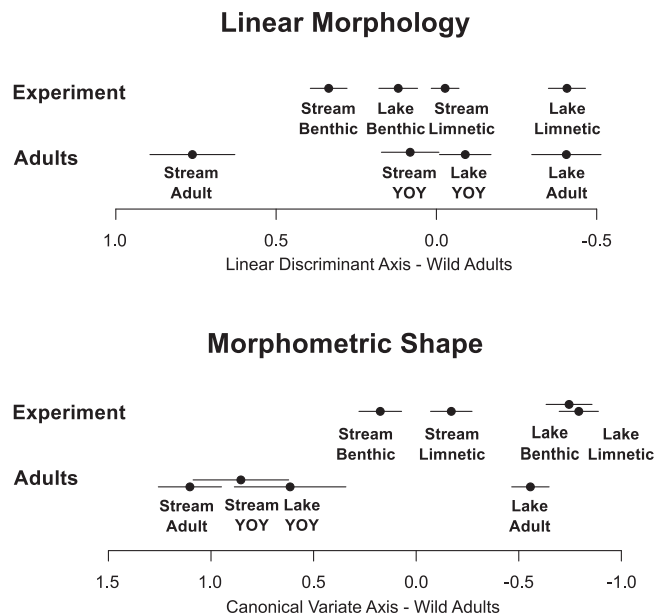

Figure 3. Average multivariate scores (± 1 SE) for the axis separating both wild-caught lake and stream individuals using either linear morphology (top) or morphometric shape data (bottom). In addition, the average scores for the wild-caught YOY individuals and the experimental groups are given, which are based on a projection onto the multivariate axis separating the wild adult (see main text for details).
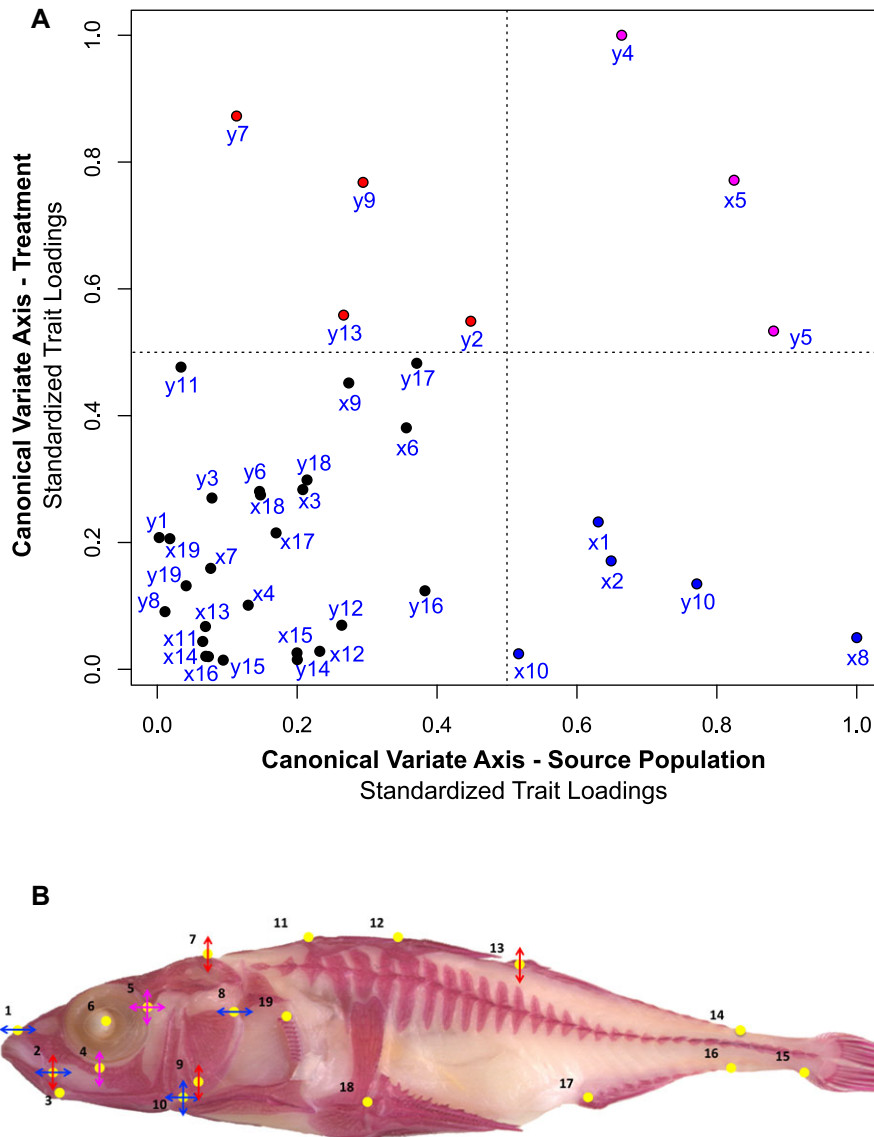
Figure 4. Differentiating between plastic and genetically determined morphometric shape traits. (A) Standardized relative trait loadings of the first canonical variate axes, calculated using either source population (lake, stream) or treatment (plankton, benthos) as grouping variable. Highlighted are traits showing high levels of genetic determination (above an arbitrary cutoff value of 0.5) and are hence either mainly genetically determined (blue), phenotypic plasticity (red), or by an interaction of both (pink). Each trait name consists of the spatial coordinates of its related landmark (i.e., x and y for changes along the horizontal or vertical axis, respectively) and the landmark ID given in (B). (B) Landmark positions with arrows indicating the shape changes associated with the highlighted traits in (A). See Table S2 for a detailed description of each landmark.

jaws from the neurocranium). The phenotypic differentiation for experimental individuals along the CV axis separating lake and stream originating individuals resulted in a similar phenotypic differentiation as observed between the wild-caught adult populations, that is, experimental lake fish had more elongated and deeper heads and longer jaws (Fig. 1D).

On the CV axis separating food treatments, traits linked to head structure as well as body shape had higher loadings, which resulted in overall shape changes along the transversal body axis. Fish raised on benthic food had deeper bodies with a larger

orbit and an increased suspensorium (Fig. 1D). Individuals raised on plankton showed a more upturned snout with the premaxilla shifted along the anteroposterior axis although with low statistical support (Table S6). The comparison of standardized loadings of the two leading axes indicated five landmarks located on the head that differ mainly between source populations, being therefore likely genetically determined. Four other landmarks showed a relatively high treatment effect and are therefore likely to be mainly driven by plasticity. These were linked to body depth (Fig. 4). In addition, three traits, all related to eye or orbit size showed an

interaction between source population and the treatment, suggesting both genetic and plastic components.

When the wild-caught populations were projected onto the CV axes that separate the experimental individuals, wild YOY from both the lake and the stream population clustered close to the experimental benthic-fed stream individuals, whereas wild-caught adults segregated toward the plankton treatment (Fig. 1B). When the wild YOY and the experimental individuals were projected onto the CV axis that separates the two wild adult populations, wild YOY from both populations clustered similarly close to the wild adults from the stream site (Fig. 3). Experimental fish that originate from the lake showed a more extreme phenotype than the wild-caught adult lake fish. Experimental stream fish on the other hand fell phenotypically in between the wild-caught adults of the two populations.

None of the statistical comparisons between the larger sample of wild adults and the individuals used to generate the experimental crosses were significant: LD scores of lake parents versus lake sampling population: $t_{1,105} = 0.07$, $P = 0.946$; LD scores of stream parents versus stream sampling population: $t_{1,60} = 1.38$, $P = 0.185$; CV scores of lake parents versus lake sampling population: $t_{1,104} = -0.88$, $P = 0.389$; CV scores of stream parents versus stream sampling population: $t_{1,52} = -0.98$, $P = 0.340$. Thus the parents used for breeding the experimental fish represent a random sample from the two populations, allowing us to infer mechanisms that underlie phenotypic differentiation along the lake–stream axis in the Lake Constance system. Finally, none of the estimated residuals from any multivariate analysis, that is, LD, PC, or CV, were statistically associated with standard length (all $P = 1.000$, results not shown). Similarly none of the MANOVA analyses that were performed using standard length as factor were statistically significant (all $P > 0.900$, results not shown). Consequently, none of the results of multivariate analyses were driven by size differences between groups.

Discussion

Evolutionary phenotypic divergence of invasive populations away from ancestral populations has commonly been found (e.g., Huey et al. 2000; Lee et al. 2003; Phillips and Shine 2006; Carroll et al. 2007; Keller and Taylor 2008; Prentis et al. 2008; Calsbeek et al. 2011; Lee et al. 2011). Similarly, yet to a lesser extent, ecotype formation between distinct habitats within a recently invaded range has been demonstrated (e.g., Hendry et al. 2000; Koskinen et al. 2002; Phillips et al. 2006; Keller and Taylor 2008; Matesanz et al. 2012, see Keller and Taylor 2008; Yoder et al. 2010 for discussion). It is however less clear if and to what extent such contemporary phenotypic evolution is triggered by phenotypic plasticity (Weinig 2000; Yeh and Price 2004; Crispo 2008; Lande 2009; Thibert-Plante and Hendry 2011), selection on stand-

ing genetic variation (Facon et al. 2006; Lee et al. 2007; Barrett and Schluter 2008) or the interaction of both. Cases of apparently rapid adaptive diversification from a single colonizing population are particularly interesting because they can be considered as a contemporary phase of what the early stages of an adaptive radiation might look like (Yoder et al. 2010). Experimental determination of the relative contribution of both of these factors can be achieved through breeding offspring from wild populations in the laboratory and rearing them in a common garden with experimental treatments that mimic a key feature of habitat contrasts between ecotypes (Kawecki and Ebert 2004). Yet, the application of this approach to simultaneously quantify both the genetic and plastic contributions to adaptive trait divergence in rapidly diverging populations has rarely been used for vertebrates (Robinson and Wilson 1996; Collyer et al. 2011) in contrast to plants (e.g., Weinig 2000; Bossdorf et al. 2005; Matesanz et al. 2012; Sultan et al. 2013) and invertebrates (e.g., Strong 1973; Carroll and Corneli 1995; Carroll et al. 2001; Lee et al. 2003). Here we applied this approach to study a case of recent parapatric ecotype formation between lake and stream habitats in invasive threespine stickleback, a species that repeatedly formed similar ecotypes after the end of the Pleistocene glaciation, ~15,000 years ago, also in its natural range (Moodie and Reimchen 1976; Reimchen et al. 1985; Hendry et al. 2002; Hendry and Taylor 2004; Berner et al. 2009; Kaeuffer et al. 2012; Ravinet et al. 2013). Hence we study the relative contributions of different evolutionary mechanisms in the very early stages of an otherwise well-studied case of ecotype formation and ecological speciation (Yoder et al. 2010).

Because we investigated the phenotypic divergence of ecotype populations in full-sib F1 offspring, maternal effects could potentially account for phenotypic divergence that is not explained by a plastic response to treatment. This seems however unlikely, given the lack of maternal care in stickleback. Maternal effects play moreover only a minor role in explaining morphological differences between ecotypes from the Misty Lake system in Canada, the only other parapatric lake–stream ecotype pair where rearing experiments were conducted (Berner et al. 2011; Table S1). Here, F2 fish reared in the laboratory were found to be phenotypically very close to F1 laboratory reared fish, which was also true for sympatric limnetic and benthic ecotypes from Paxton Lake, Canada (Hatfield 1997). Therefore, our experimental and statistical design is suited to quantify for each investigated trait the contributions of phenotypic plasticity and genetic predisposition to ecotypic divergence, and given the limited influence of maternal effects on morphology in other stickleback populations, we think it is unlikely that maternal effects account for the predisposition.

Overall we find the phenotypic differentiation among our experimental groups to be similar with that observed between the adult wild lake–stream populations, as suggested by the

pairwise Mahalanobis distances and the relative classification success (Figs. 1, 3). We estimate the relative contribution of both phenotypic plasticity and genetic predisposition responsible for the phenotypic divergence between wild lake and stream populations using a novel approach that in principle is widely applicable when studying rapid ecotype formation between sister taxa (Fig. 4), that is, by comparing trait loadings on the major multivariate axes separating either only the source populations or the experimental treatment (food) of our fish. For both wild and experimental fish, differentiation occurs mainly in functionally relevant trophic morphology that is predicted to facilitate exploiting alternative habitats and resources (Anker 1974; Walker 1997; Robinson 2000; Wark and Peichel 2010; McGee et al. 2013). This suggests that contemporary ecotype formation has occurred as a consequence of divergent adaptation to different habitats and adaptive phenotypic plasticity (Fig. 3, Ghalambor et al. 2007). This is apparent when experimental and YOY individuals were projected onto the axis that separates the wild adult populations (Fig. 3). All projected groups follow the predicted direction, underlying the importance of the combined effect of phenotypic plasticity and additive genetic variation that leads to overall phenotypic divergence. For morphometric shape, experimental individuals deriving from the lake even exceed the wild-type adult lake phenotype. This may indicate limitations of our experimental setup because we focus on a single, albeit important axis of parapatric population divergence, that is, on feeding ecology. Additional differences between lake and stream habitats may result in the overall observed phenotypic divergence among the wild adult populations.

PHENOTYPIC DIVERGENCE IN WILD AND EXPERIMENTAL POPULATIONS

The observed phenotypic differentiation among our studied wild populations is in line with former evidence for ecotypic differentiation in the same populations (Roy et al. 2010; Lucek et al. 2012, 2013), and more generally within the Lake Constance region (Berner et al. 2010). Ontogenetic trajectories are likely to differ given the inverse size difference among YOY and adults in the wild, which has previously been suggested (Lucek et al. 2012; Moser et al. 2012). The observed increase in body depth among wild stream fish is thought to be associated with increased maneuverability and burst swimming, required for predator avoidance in structured habitats, whereas a smaller body depth in lake fish may facilitate sustained swimming performance, facilitating foraging in open water (Walker 1997; Wark and Peichel 2010; Hendry et al. 2011). Other common and adaptive features of lake dwelling and plankton feeding stickleback ecotypes are elongated heads with larger eyes and longer gill rakers (Robinson 2000; Wund et al. 2008; Willacker et al. 2010). Differentiation between lake and stream stickleback in these traits occurs already in YOY, but is overall less developed than in adults (Fig. 1).

Our experiment suggests that the ecotypic differentiation in head shape and trophic morphology is substantially genetically determined, whereas differentiation in body depth is mainly environmentally induced, with fish from the plankton treatment being more streamlined than fish from the benthos treatment, irrespective of source population (Figs. 1D, 4A). The additive effects of both source populations and treatments result in similar phenotypic differentiation as found in the wild populations (Fig. 3). This suggests that adaptation to different food sources is an important driver of phenotypic divergence in the wild. Moreover, it implies that the concerted action of divergent genetic predisposition and adaptive plasticity can lead to the onset of ecological diversification (Prentis et al. 2008; Yoder et al. 2010; Thibert-Plante and Hendry 2011; Westley 2011).

In contrast to morphometric shape, environmentally induced changes are the main contributors of the observed phenotypic variation using linear morphology (Fig. 2), where the significant interactions for PC scores suggest different multivariate reaction norms between the source populations (Proulx and Magnan 2004). This is a common finding among studies using linear morphology to investigate ecotype formation in postglacial freshwater fish (Robinson and Wilson 1996; Adams and Huntingford 2004; Proulx and Magnan 2004). The differences between morphometric shape and linear morphology may reflect the distinctive way in which the covariance structure was calculated for each type of phenotypic data. Furthermore, each approach may differ in its ability to isolate size effects and hence to disentangle heritable or plastic effects on size-dependent traits or on size itself.

Our results contrast in an interesting way with studies on postglacial ecotype formation in the Misty Lake system, the only other parapatric lake–stream stickleback ecotype pair where rearing experiments were performed (Table S1). Ecotypic differentiation in body shape was found to be genetically determined in the Misty Lake system (Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008; Berner et al. 2011), whereas the shape of the snout was relatively plastic (Berner et al. 2011). Several factors may account for these differences. First, the adaptive potential and genetic constraints of the ancestral populations may differ between our studied ecotype pair and the Misty Lake system, resulting in different evolutionary responses to similar selection (Jones et al. 2012). Indeed, the traits that underlie lake–stream ecotype divergence in Switzerland were found to differ from those in the Misty system. Specifically, the Misty lake population has a higher number of gill rakers than the stream population, and the same is true for other lake–stream ecotype pairs in British Columbia, but this is not the case in Switzerland, where lake populations have longer gill rakers but not more gill rakers than stream populations (Kaeuffer et al. 2012; Lucek et al. 2013; Ravinet et al. 2013). Second, the colonization history differs between the Misty Lake and the Lake Constance system. Whereas Lake Misty was colonized

directly from the Sea, and possibly on two independent occasions (Thompson et al. 1997), Lake Constance was only colonized once and most likely by stickleback with a history in freshwaters (Lucek et al. 2010). Thus both, genetic variation and ancestral plasticity may have been different for different traits in the Misty and the Constance stickleback, which would likely lead to different trajectories of phenotypic divergence (West-Eberhard 2003; Thibert-Plante and Hendry 2011). Third, the selective regime is likely to be different too because Lake Misty is rather small and shallow and only holds few other fish species (Lavin and McPhail 1993). Lake Constance in contrast is an order of magnitude larger and deeper and contains a rich community of at least 30 different fish species (Reyjol et al. 2005). Lastly, if ecotype formation in stickleback is associated with phenotypic canalization or shifts in the reaction norms, divergent genetic determination of body depth may not yet have occurred in the young pair of Lake Constance. Plasticity can be maintained if it is not costly or when selection is relatively weak or fluctuating (Lande 2009; Thibert-Plante and Hendry 2011).

ADAPTIVE DIVERGENCE

Differences between environments can induce divergent selection on functional and biomechanical traits, resulting in ecological divergence (Nosil et al. 2009). The adaptive value of our distinct lake and stream phenotypes can be assessed through comparisons with other stickleback ecotypes and fish species with analogous adaptations (Barel 1983; Caldecutt and Adams 1998; McGee et al. 2013). The observed shape differences in both experimental and wild-caught individuals have consequently functional morphological implications related to feeding: the terminal mouth in wild and laboratory reared lake sticklebacks together with an anterior shift of the maxilla and a smaller suspensorium are predicted to result in a reduction of suction force compared to the anatomy of wild and laboratory-reared stream sticklebacks (Caldecutt and Adams 1998). In addition, in cichlid fish, limnetic plankton feeding species tend to generally have elongated and slender heads (Barel 1983). This was observed in our experimental plankton treatment, irrespective of source population, and in both age classes of the wild-type lake population, where individuals showed decreased head depth and a terminal mouth relative to individuals from either the benthic treatment or the wild-type stream population. Together, these differences result in phenotypes that may be more suitable for ram-type feeding in lake fish compared to more suction-type feeding in stream fish (Caldecutt and Adams 1998) predicting fitness advantages for each ecotype in its own environment (Lucek et al. 2012).

The observed phenotypically plastic difference for experimental individuals in body depth, with limnetic-fed fish being more streamlined than their benthic-fed counterparts, is consistent with the phenotypic differentiation between the wild adult populations, which feed either predominantly on limnetic prey in the lakes or on benthic prey in streams (Lucek et al. 2012; Moser et al. 2012). The more streamlined plankton feeding phenotype is furthermore in line with observations in other wild stickleback populations that differ along the benthic–limnetic axis (Walker 1997; Hendry and Taylor 2004; Willacker et al. 2010) and may result in an increased sustained swimming capability (Walker 1997; Blake et al. 2005). Indeed, the observed plastic response in body depth between our experimental food treatments could be caused by different foraging behavior as swimming effort differed between treatments, that is, feeding on live zooplankton required an increased sustained swimming capability compared to feeding on benthic insect larvae. Taken together, the observed phenotypic differences are consistent with additive effects of adaptive plasticity and divergent adaptation. Because former genetic analyses showed that the lake and stream populations investigated here are very closely related, and in fact are more closely related to each other than to other Swiss populations (Berner et al. 2010; Lucek et al. 2010, 2013), the lake–stream divergence observed here likely evolved in situ. Hence, we have shown ecotypic differentiation that has evolved within less than 140 years among populations of an invasive species that occupy distinct habitats.

The observed and potentially heritable phenotypic differentiation related to habitat and feeding ecology mark the transition from invasion and niche expansion with establishment in a new environment toward populations that undergo divergent adaptation (Hendry et al. 2000; Prentis et al. 2008). Such adaptive evolutionary divergence might be a precursor of ecological speciation (Nosil 2012). Phenotypic plasticity may, on the other hand, delay or even prevent further divergence by shielding the genome from divergent selection depending on the underlying selective regime (Price et al. 2003; Ghalambor et al. 2007; Thibert-Plante and Hendry 2011). The formation of divergent ecotypes can have further implications for the impact of the invasive species on native species and the ecosystem itself: By adapting to effectively exploiting different niches, different stickleback ecotypes are likely to introduce different selection pressures on their prey, competitors, and predators and may hence induce divergent evolutionary responses in other organisms (Vellend et al. 2007; Shine 2012). Experimental evidence suggests that divergent stickleback ecotypes from within the native range are indeed able to affect the community composition of lower trophic levels in distinct ways (Harmon et al. 2009). However, further studies are needed to assess the generality of our findings as Lake Constance stickleback provide only the second case for which rearing experiments were used to study parapatric lake–stream divergence (Table S1).

valuable comments on earlier versions of the manuscript. M. Lemoine provided valuable statistical assistance for linear models. M. P. Haesler and M. Zeller helped capturing the wild fish in 2010 as well as feeding the experimental fish. This study was supported by an EAWAG Action field grant "AquaDiverse."

DATA ARCHIVING

The doi for our data is 10.5061/dryad.rh686.

LITERATURE CITED

Abràmoff, M., P. Magalhães, and S. Ram. 2004. Image processing with Image J. Biophot. Int. 11:36–42.

Adams, C. E., and F. A. Huntingford. 2004. Incipient speciation driven by phenotypic plasticity evidence from sympatric populations of arctic charr. Biol. J. Linn. Soc. 81:611–618.

Anker, G. C. 1974. Morphology and kinetics of the head of the stickleback, *Gasterosteus aculeatus*. Trans. Zool. Soc. Lond. 32:311–416.

Barel, C. D. N. 1983. Towards a constructional morphology of cichlid fishes (Teleostei, Perciformes). Neth. J. Zool. 33:357–424.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends Ecol. Evol. 23:38–44.

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

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

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

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

Blake, R. W., T. C. Law, K. Chan, and J. Li. 2005. Comparison of the prolonged swimming performances of closely related, morphologically distinct three-spined sticklebacks *Gasterosteus* spp. J. Fish. Biol. 67:834–848.

Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11.

Caldecutt, W., and D. Adams. 1998. Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. Copeia 1998:827–838.

Calsbeek, B., S. Lavergne, M. Patel, and J. Molofsky. 2011. Comparing the genetic architecture and potential response to selection of invasive and native populations of reed canary grass. Evol. Appl. 4:726–735.

Carroll, S. P., and S. P. Corneli. 1995. Divergence in male mating tactics between two populations of the soapberry bug: II. Genetic change and the evolution of a plastic reaction norm in a variable social environment. Behav. Ecol. 6:46–56.

———. 1999. The evolution of behavioral norms of reaction as a problem in ecological genetics. Pp. 52–68 *in* S. A. Foster and J. Endler, eds. Geographic variation in behavior: perspectives on evolutionary mechanisms. 1st ed. Oxford Univ. Press, New York, NY.

Carroll, S. P., H. Dingle, T. R. Famula, and C. W. Fox. 2001. Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma*. Genetica 112–113:257–272.

Carroll, S. P., A. P. Hendry, D. N. Reznick, and C. Fox. 2007. Evolution on ecological time-scales. Funct. Ecol. 21:387–393.

Collyer, M. L., J. S. Heilveil, and C. A. Stockwell. 2011. Contemporary evolutionary divergence for a protected species following assisted colonization. PLoS One 6:e22310.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J. Evol. Biol. 21:1460–1469.

Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. Evolution 48:1723–1734.

Facon, B., B. J. Genton, J. Shykoff, P. Jarne, A. Estoup, and P. David. 2006. A general eco-evolutionary framework for understanding bioinvasions. Trends Ecol. Evol. 21:130–135.

Ghalambor, C. K., J. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21:394–407.

Harmon, L. J., B. Matthews, S. Des Roches, J. M. Chase, J. B. Shurin, and D. Schluter. 2009. Evolutionary diversification in stickleback affects ecosystem functioning. Nature 458:1167–1170.

Hatfield, T. 1997. Genetic divergence in adaptive characters between sympatric species of stickleback. Am. Nat. 149:1009–1029.

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

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

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

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

Huey, R., G. W. Gilchrist, M. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science 287:308–309.

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

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

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

Keller, S. R., and D. R. Taylor. 2008. History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol. Lett. 11:852–866.

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

Koskinen, M. T., T. O. Haugen, and C. R. Primmer. 2002. Contemporary fisherian life-history evolution in small salmonid populations. Nature 419:826–830.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. J. Evol. Biol. 22:1435–1446.

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

Lee, C. E., J. L. Remfert, and G. Gelembiuk. 2003. Evolution of physiological tolerance and performance during freshwater invasions. Integr. Comp. Biol. 43:439–449.

Lee, C. E., J. L. Remfert, and Y.-M. Chang. 2007. Response to selection and evolvability of invasive populations. Genetica 129:179–192.

Lee, C. E., M. Kiergaard, G. W. Gelembiuk, B. D. Eads, and M. Posavi. 2011. Pumping ions: rapid parallel evolution of ionic regulation following habitat invasions. Evolution 65:2229–2244.

Lemoine, M., B. Doligez, and H. Richner. 2012. On the equivalence of host local adaptation and parasite maladaptation: an experimental test. Am. Nat. 179:270–281.

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

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

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

Matesanz, S., T. Horgan-Kobelski, and S. E. Sultan. 2012. Phenotypic plasticity and population differentiation in an ongoing species invasion. PLoS One 7:e44955.

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

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in the Enos Lake species pair. Can. J. Zool. 62:1402–1408.

Moodie, G., and T. E. Reimchen. 1976. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Syst. Zool. 25:49–61.

Moser, D., M. Roesti, and D. Berner. 2012. Repeated lake-stream divergence in stickleback life history within a Central European lake basin. PLoS One 7:e50620.

Nosil, P. 2012. Ecological speciation. 1st ed. Oxford Univ. Press, Oxford, U.K.

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

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

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

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

Phillips, B. L., G. P. Brown, J. K. Webb, and R. Shine. 2006. Invasion and the evolution of speed in toads. Nature 439:803.

Prentis, P. J., J. R. U. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008. Adaptive evolution in invasive species. Trends Plant Sci. 13:288–294.

Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B 270:1433–1440.

Proulx, R., and P. Magnan. 2004. Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.). Evol. Ecol. Res. 6:503–522.

R Core Team. 2012. R 2.15.1. R Foundation for Statistical Computing, Vienna, Austria.

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

Reimchen, T. E., E. M. Stinson, and J. S. Nelson. 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.

Reyjol, Y., P. Fischer, S. Lek, R. Rosch, and R. Eckmann. 2005. Studying the spatiotemporal variation of the littoral fish community in a large prealpine lake, using self-organizing mapping. Can. J. Fish. Aquat. Sci. 62:2294–2302.

Reznick, D. N., and J. Endler. 1982. The impact of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177.

Reznick, D. N., and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112:183–198.

Robinson, B. 2000. Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. Behaviour 137:865–888.

Robinson, B. W., and D. S. Wilson. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evol. Ecol. 10:631–652.

Rohlf, F. J. 2006. Version 2.10. Department of Ecology and Evolution, State University, Stony Brook, New York.

Roy, D., K. Lucek, E. Bühler, and O. Seehausen. 2010. Correlating shape variation with feeding performance to test for adaptive divergence in recently invading stickleback populations from Swiss peri-alpine environments. Lect. Notes Earth Sci. 124:233–257.

Sakai, A., F. Allendorf, J. Holt, D. Lodge, J. Molofsky, K. With, S. Baughman, R. Cabin, J. Cohen, N. C. Ellstrand, et al. 2001. The population biology of invasive species. Annu. Rev. Ecol. Evol. Syst. 32:305–332.

Schluter, D. 2000. The ecology of adaptive radiation. 2nd ed. Oxford Univ. Press, Oxford, U.K.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. Am. Nat. 140:85–108.

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

Shine, R. 2012. Invasive species as drivers of evolutionary change: cane toads in tropical Australia. Evol. Appl. 5:107–116.

Simpson, G. G. 1953. The major features of evolution. 1st ed. Columbia Univ. Press, New York.

Strong, D. R. Jr. 1973. Amphipod amplexus, the significance of ecotypic variation. Ecology 54:1383–1388.

Sultan, S. E., T. Horgan-Kobelski, L. M. Nichols, C. E. Riggs, and R. K. Waples. 2013. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. Evol. Appl. 6:266–278.

Svanbäck, R., and D. Schluter. 2012. Niche specialization influences adaptive phenotypic plasticity in the threespine stickleback. Am. Nat. 180:50–59.

Thibert-Plante, X., and A. P. Hendry. 2011. The consequences of phenotypic plasticity for ecological speciation. J. Evol. Biol. 24:326–342.

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

Vellend, M., L. J. Harmon, J. L. Lockwood, M. M. Mayfield, A. R. Hughes, J. P. Wares, and D. F. Sax. 2007. Effects of exotic species on evolutionary diversification. Trends Ecol. Evol. 22:481–488.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. 4th ed. Springer, New York.

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

Wark, A. R., and C. L. Peichel. 2010. Lateral line diversity among ecologically divergent threespine stickleback populations. J. Exp. Biol. 213:108–117.

Weinig, C. 2000. Plasticity versus canalization: population differences in the timing of shade-avoidance responses. Evolution 54:441–451.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. 1st ed. Oxford Univ. Press, Oxford, U.K.

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.

Willacker, J. J., F. A. von Hippel, P. R. Wilton, and K. M. Walton. 2010. Classification of threespine stickleback along the benthic-limnetic axis. Biol. J. Linn. Soc. 101:595–608.

Wund, M. A., J. A. Baker, B. Clancy, J. L. Golub, and S. A. Foster. 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.

Yeh, P. J., and T. D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. Am. Nat. 164:531–542.

Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, et al. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. 23:1581–1596.

Associate Editor: C. Lee

Supporting Information

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

Figure S1. Phenotypic differentiation between wild-caught adults (left) and young of the year (YOY; right) for linear morphology based on linear discriminant scores (top) and morphometric shape differentiation based on canonical variate scores (bottom).

Figure S2. Family means ($\pm$ 1 SE) for all linear traits measured including gill raker number and the three leading principal component axes separated by treatments (benthos and plankton) and source populations (lake and stream).

Figure S3. Relative trait loadings of the leading linear discriminant axis based on linear morphological data from experimental individuals using either source population (lake, stream) or treatment (plankton, benthos) as grouping variable.

Figure S4. Morphometric shape differences along the three leading principal component axes for experimental individuals.

Table S1. Comparison of studies that use rearing experiments to investigate ecotype formation in freshwater threespine stickleback (*Gasterosteus aculeatus* species complex).

Table S2. Description of the landmarks used for shape analysis.

Table S3. Loadings based on linear discriminant analyses for each linear measurement for wild-caught adults, young of the year (YOY), and experimental individuals.

Table S4. Significances for linear measurements for both wild-caught adults and young of the year (YOY) based on pairwise *t* tests.

Table S5. *P*-values for all linear size-corrected morphological traits separately and combined using principal components (PC; axes 1–3 indicated).

Table S6. Standardized relative loadings for each landmark coordinate based on canonical variate analyses.