Title: Genetic and phenotypic population divergence on a microgeographic scale in brown trout.
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Journal: Molecular Ecology
Year: 2012

Volume: 21(12)

Pages: 2896-2915

DOI: 10.1111/j.1365-294X.2012.05581.x

Genetic and phenotypic population divergence on a microgeographic scale in brown trout

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Abstract

Salmonid populations of many rivers are rapidly declining. One possible explanation is that habitat fragmentation increases genetic drift and reduces the populations' potential to adapt to changing environmental conditions. We measured the genetic and eco-morphological diversity of brown trout (*Salmo trutta)* in a Swiss stream system, using multivariate statistics and Bayesian clustering. We found large genetic and phenotypic variation within only 40 km of stream length. Eighty-eight percent of all pairwise F_{ST} comparisons and 50% of the population comparisons in body shape were significant. High success rates of population assignment tests confirmed the distinctiveness of populations in both genotype and phenotype. Spatial analysis revealed that divergence increased with waterway distance, the number of weirs, and stretches of poor habitat between sampling locations, but effects of isolation-by-distance and habitat fragmentation could not be fully disentangled. Stocking intensity varied between streams but did not appear to erode genetic diversity within populations. A lack of association between phenotypic and genetic divergence points to a role of local adaptation or phenotypically plastic responses to habitat heterogeneity. Indeed, body shape could be largely explained by topographic stream slope, and variation in overall phenotype matched the flow regimes of the respective habitats.

Keywords: habitat fragmentation, isolation-by-distance, landscape genetics, local adaptation, phenotypic plasticity, *Salmo trutta*

Introduction

Natural populations around the globe are increasingly exposed to habitat-altering activities of humans (Kinnison *et al.* 2008; Smith & Bernatchez 2008) to the extent that, in some environments, humans have become the most important driver of phenotypic change (Hendry *et al.* 2008). Strong anthropogenic impacts on ecosystems can pose serious threats to biodiversity. One of the most affected species groups are fish (Taylor *et al.* 2006; Meeuwig *et al.* 2008). Salmonid populations are in decline worldwide for reasons often unidentified but usually related to their commercial over-exploitation, the extensive agricultural use of streams and lakes, and energy generation (McGinnity *et al.* 2003; ICES 2006; Krkosek *et al.* 2007; Ford & Myers 2008). One of the most common and problematic consequences for natural populations is the fragmentation of habitat, which often causes increased genetic drift. An erosion of genetic variation and the loss of rare alleles and traits can severely constrain the adaptability of populations, especially when environmental conditions change quickly (Ferguson 1989; Hedrick & Miller 1992; Meldgaard *et al.* 2003). If fragmentation is extreme and persists, inbreeding can lead to the fixation of deleterious alleles reducing the fitness of populations, e.g. by decreasing survival and fertility (Frankham 1995; Hedrick & Kalinowski 2000; Armbruster & Reed 2005; Charlesworth & Willis 2009). Especially when populations are small, environmental or demographic stochasticity can become the principal threat driving entire populations to extinction (Lande 1992).

The use of geographical landscape data paired with population genetics or genomics has become a popular and efficient tool to interpret the effects of landscape structures on population connectivity. Landscape genetics seeks to understand how the interaction between landscape features and evolutionary processes - such as gene flow, genetic drift, and selection - shapes the spatial distribution of genetic variation (Manel *et al.* 2003; Cushman *et al.* 2006; Holderegger & Wagner 2006; Lowry 2010; Manel *et al.* 2010; Storfer *et al.* 2010). Salmonid species make an interesting case for investigating the effects of habitat fragmentation on population structure because the linear network of streams and lakes they inhabit makes the identification of dispersal corridors straightforward.

Genetic differentiation in freshwater salmonids can occur across short geographic distances. On the one hand, the accurate natal homing abilities of many species (Neville *et al.* 2006b) and the limited migratory behaviour of resident populations (Northcote 1992) may be sufficient to maintain genetic differentiation, even in the absence of physical barriers to dispersal (Carlsson & Nilsson 2000; Griffiths *et al.* 2009). In addition, diversifying ecological selection can lead to local adaptation and drive populations to diverge (Taylor 1991; Dionne *et al.* 2008). On the other hand, natural landscape features like waterfalls and tributary networks (Kanno *et al.* 2011), but also artificial structures such as dams, weirs and gradation steps, may constrain gene flow and promote population differentiation through drift (Meldgaard *et al.* 2003). Brown trout (*Salmo trutta*) can show especially complex population structure, often with substantial genetic differentiation within river catchment. Another feature characteristic of brown trout populations is their vast phenotypic diversity (e.g. Hermida *et al.* 2009) and large variation in life history strategies (Nielsen *et al.* 2003). This variation has, since 1758, led to ca. fifty different species descriptions for brown trout (Ferguson 1989).

Brown trout catches in the Aare river, Switzerland, have decreased by more than 50% over the past 30 years, indicative of a dramatic population decline (Fischnetz 2004; Burkhardt-Holm 2007). Although well documented, a conclusive explanation for the decline is lacking (Burkhardt-Holm *et al.* 2002; Borsuk *et al.* 2006; Zimmerli *et al.* 2007). Artificial landscape structures such as culverts (subterranean canalization of streams, usually through concrete pipes), embankments, irrigation, dams, weirs and grade control structures are ubiquitous in the Aare catchment. Recent fine-scale mapping of Swiss midland streams showed that 2.5 barriers (of >50cm height) interrupt every kilometre of stream, and the total length of artificial stream sections in Switzerland amount to a third of the Earth's circumference (14,000km; (Zeh-

Weissmann *et al.* 2009).

Here, we provide a geographically fine-scaled investigation of the influence of habitat fragmentation on the genetic and phenotypic composition of brown trout populations in the river Aare, a drainage system typical for the Swiss prealpine and midlands. We investigate 1) the genetic diversity of brown trout present in the catchment, 2) their phenotypic diversity in multivariate body shape, 3) the impact of isolation-by-distance, dispersal barriers, topographic stream slope and stocking on diversity, and 4) the association of genetic and phenotypic divergence.

Methods

Sampling of biological material

Between June and October 2009, brown trout from 21 locations were collected from the main stem and the tributaries of the river Aare between Lake Thun and the city of Bern (Figure 1, for sample sizes per site see Table 1). The number and size of all fish that could be collected by wading and electrofishing upstream a 100m stretch were recorded. For analysis purposes and simplicity, each site is assumed to be a 'population', representing a sample and not necessarily a biological population. Of those individuals that, from the observed size distribution, could be assumed as > 1+ in age, a random sample of a total of 603 *S. trutta* was taken and given the following treatment: After light anesthetization (0.5 ml/L Koi Med Sleep; Koipraxis, Ulmiz, Switzerland) a digital photograph was taken of the right body side with a size scale for later allometric adjustment. A tissue sample was taken from the pelvic fin and preserved in 99.9% ethanol. After sufficient time to recover in a freshwater tank, all individuals were released at the site of capture.

Microsatellite amplification

Genomic DNA was extracted using the DNeasy tissue kit (Qiagen) following instructions of the manufacturer. Individuals were genotyped at 11 microsatellite loci: *BS131*, *T3-13* (Estoup *et al.* 1998), *Mst543AE*, *Str591INRA* (Presa & Guyomard 1996), *Ssa171* (O'Reilly *et al*. 1996), *Omy5*, *Str12INRA* (Gharbi *et al*. 2006), *AETG1*, *Str15INRA* (Estoup *et al.* 1993), *Sssosl41* (Slettan *et al.* 1995), *Str58CNRS* (Poteaux *et al*. 2000), according to the protocol in Jacob *et al.* (2007). PCR reactions were done in three multiplex reactions (multiplex 1: *BS131, Mst543AE, Ssa171, Omy5;* multiplex 2: *AETG1, Str58CNRS, T3-13, Sssosl41;* multiplex 3: *Str15INRA, Str591INRA, Str12INRA*). PCR profiles were 35 iterations of 95°C for 15min, 94°C for 30s, 59°C (mix 1) or 57°C (mix 2) or 56°C (mix 3) for 1:30min, 72°C for 1min, and a final extension at 60°C for 30min. PCR products were typed on an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA, USA) and allele lengths were sized using GeneMapper 3.7 (Applied Biosystems). 20% of the individuals were genotyped at least twice at the same locus to estimate genotyping error (Hoffman & Amos 2005). Systematic error and stochastic error were smaller than 1% for all loci.

Genetic variability analysis

Nuclear genetic variability within populations was quantified by the mean number of alleles across loci (N), allelic richness (k), observed heterozygosity (H_O), and expected heterozygosity within populations (H_E) using FSTAT 2.9.4 (Goudet 2002) (Table 1). Linkage disequilibrium between all pairs of loci was calculated based on 1,100 permutations. Wright's fixation index for within-population variation per locus (i.e. deviation from random mating, F_{IS}) was obtained using permutation. At some of the Aare main stream sites, not enough specimens could be collected to justify the use of any estimate of genetic variation. Sites 203.1 (n = 2) 205.1 (n = 0) and 205.2 (n = 11) were therefore excluded from all further analyses, leaving a total of 590 specimens. Populations 203.2 and 203.3, and populations 208.1, 208.2 and 208.3 were grouped for analysis since they were geographically close.

Global genetic diversity (F_{ST}) and pairwise population differentiation were computed (Weir & Cockerham 1984), and confidence intervals obtained by randomizing multi-locus genotypes between pairs of samples with 20,000 bootstrap permutations (Table 2). Critical significance levels were obtained using sequential Bonferroni corrections (Rice 1989). Interpretation of the genetic differentiation measure F_{ST} can be problematic because F_{ST} depends on the level of mean heterozygosity within populations and necessarily declines with increasing polymorphism (Hedrick 2005; Jost 2008; Gerlach *et al.* 2010). To overcome this problem, we also used the 'actual differentiation' estimator D_{est} (Jost 2008), a standardized measure of genetic differentiation with the same range (0–1) for all levels of genetic variation independent of average within-subpopulation gene diversity (Table 2). Roughly, D_{est} applies a weighting treatment where populations are weighted by both their size and their average within-subpopulation heterozygosity. D_{est} calculations were performed using SMODG 1.2.5 (Crawford 2010) (see accordance between F_{ST} and D_{est} estimates in Figure S1).

The genetic structure of the 13 tributary populations was further investigated using the Bayesian statistical framework implemented in the software STRUCTURE 2.3.3 (Pritchard *et al.* 2000; Falush *et al.* 2007) executed on the freely available bioinformatics service 'Bioportal', University of Oslo, Norway (http://www.bioportal.uio.no). STRUCTURE evaluates the most likely structure without prior information of the population affiliation of each individual and provides an unbiased estimate of the structure with respect to the sampled populations. We used a burn-in period of 100,000 iterations and a sampling period of 500,000 iterations in admixture models (where a fraction of the genome of each individual is equally likely to originate from each population under consideration). We performed runs for one to 15 clusters (K, the putative number of biological populations) with six iterations for each K to quantify the variation in likelihood, and calculated the logarithm of the mean posterior probability of the data L(K). To identify the most likely number of K, we used the maximal value of L(K) returned by STRUCTURE (e.g. Zeisset & Beebee 2001) and calculated the ΔK statistic using the second order rate of change in log probability between successive K values following Evanno *et al*. (2005).

To estimate the number of individuals correctly assigned to their sample populations based on multivariate molecular data, we carried out a discriminant function analysis on principal components of allelic data from 11 microsatellites (DAPC; Jombart *et al.* 2010), using the R package *adegenet* (Jombart 2008). Feeding PCs into a discriminant function analysis (DA) holds the crucial advantage that all variables are uncorrelated.

Geometric morphometric analysis of shape

Of the 590 sampled individuals, 581 were phenotyped for body shape using landmark-based geometric morphometrics methods (photos of 9 individuals were of too low quality). The position of the 13 landmarks (Figure 2) were chosen based on previously published work on trout morphometrics (Varian & Nichols 2010). Landmarks were placed on standardized digital pictures on the right body side of each fish. Geometric morphometric analysis was performed on the XY coordinates of these landmarks using the software tpsDig2.10 according to the protocol described in Stelkens *et al*. (2009). Non-shape variation, introduced through variation in position, orientation, and size, was mathematically removed using generalized procrustes analysis (GPA; Rohlf & Slice 1990). GPA superimposes landmark configurations by minimizing the sum of squared distances between corresponding landmarks by scaling, translating and rotating specimens onto a mean consensus configuration calculated from all specimens. To obtain partial warps, thin-plate spline (TPS) procedure was applied using the software tpsRelw2.10. Partial warps estimate the minimum bending energy needed to deform an infinitely thin metal plate (i.e. the landmark configuration of an individual fish) to adopt the shape of another landmark configuration (i.e. the consensus configuration of all fish) while being constrained at particular points (i.e. the landmarks).

Among-population shape differences can also be caused by shearing and stretching, affecting all landmarks at once. These shape variables are called uniform components. To test if there was significant variation between populations in uniform components, we applied MANOVA using population as factor and the uniform components as response variables in JMP9 (SAS). The analysis revealed significant differentiation between populations in uniform components, which were therefore included in all downstream analyses. All partial warps were regressed against size (a product of the size of each individual and the centroid size) to remove potential allometry effects. Residuals of each of these regressions were extracted and principal component analysis (PCA) was used, including all partial warp residuals and the uniform components, to identify the major axes of shape variation. After PCA, principal components explaining more than 10% of the variance (PC1 and PC2) were maintained for downstream analysis. We then carried out one-way ANOVAs using population as factor and each PC as response variable to test if populations show significant variance in major body shape components. Deformation grids in Figure 3 were obtained using the software tpsReg1.3, performing multivariate multiple regression of shape (captured by partial warp scores and the uniform shape components) onto PC1 and PC2, respectively. Posthoc Tukey-Kramer tests were used for pairwise population comparisons. Canonical linear discriminant analysis was used to explore if individuals could be correctly reassigned to their source group based on principal components of shape.

Phenotypic variability analysis

The divergence in quantitative phenotypic traits between populations was estimated by calculating pairwise P_{ST} estimates (the phenotypic surrogate for $Q_{ST;}$ (Spitze 1993)). Because landmarks and associated morphometric distances are non-independent, P_{ST} values were estimated from the first two principal components (PC1 and PC2). All analyses were run in R (R 2006). Calculations were based on: $P_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$ with σ^2_{GB} as the average variance component among populations, and σ^2_{GW} as the average variance component within populations (Lande 1992; Spitze 1993).

Sampling of landscape variables

Waterway distances between sampling sites and putative barriers to trout dispersal were extracted from geographic information system (GIS) data available from the 'Geoportal', an interactive geo-referenced map maintained by the government of the Bern canton, Switzerland (http://www.apps.be.ch/geo). Data were analysed in the software ArcGIS10. Although not listed on Geoportal, the only two weirs (Schwellenmätteli and Engehalde) present in the Aare main stem, located between the collection sites Aare 205.3 and 208.1, were also included in the analysis. Waterway distance (*geoD*) was measured in km. For distances between tributary populations and the three Aare main stem groups, the geographically closest sampling location in the Aare was chosen. Barriers were identified and categorized in the following way. First, we used the total number of barriers between populations regardless of their height and type (*nbarr,* including waterfalls, weirs, buildings, gradation steps and other structures listed on Geoportal). Second, this measure was further separated into the total number of natural (*natbarr*), and artificial barriers (*artbarr*). We also tested for effects of barrier frequency between populations, calculated as *nbarr* divided by *geoD* (*freqbarr*).

Some consider only drops of at least 180cm as large enough to constrain upstream movement (Meeuwig *et al.* 2010), while others regard drops of 20-70cm (BUWAL 2005), or at least 50cm (Zeh-Weissmann *et al.* 2009) as sufficient to restrict dispersal. We thus divided *nbarr* into four different height categories of barriers using information provided on Geoportal (*nbarr30*$\leq$ 30cm, *nbarr80* = 31-80cm, *nbarr150* = 81-150cm, *nbarr150*+$\geq$ 151cm).

We also evaluated the number of weirs (*nweirs*), and the number of buildings along the stream (*nbuild*), separately. By definition, 'weirs' are structures built more or less perpendicular

to the water flow within the water column. They stretch across the entire width of the stream but allow water to flow over the top. 'Buildings' can be bridges or houses built above the water level, but also structures like sluices, ramps or chutes built on the sides or the bottom of the stream below water level, so that water flow may be altered but not as strongly interrupted as through weirs.

Further, we tested for the impact of habitat stretches between populations that were categorized as 'artificial' or 'culvert' on Geoportal. We measured the total length of culverts (*cullength*) and sections of artificial habitat (*arthab*) between pairs of populations. Because every 150m of artificial habitat and every 70m of culverts are considered equally inhibitive to gene flow as one physical barrier (Zeh-Weissmann *et al.* 2009), *arthab* and *cullength* were divided by 150 and 70, respectively, and added to the total number of barriers (*nbarrculart*) to obtain an overall measure of the fragmentation of the dispersal routes between populations.

Besides the amount of barriers, the streams in this study also differ substantially in their topographic slope gradients. Some streams originate in steep areas of the pre-Alps (e.g. Zulg) while others run in valleys (e.g. Gürbe) or close and parallel to the Aare (Giesse Münsingen, Giesse Belp; see Figure 1). To account for this difference in stream ecology and the resulting variation in trout habitat, we measured the elevation 2km up- and downstream from each sampling site (Table 1), using Google Earth. The difference in elevation was divided by the total distance between the two reference points (4km) to obtain a measure of the slope of the stream.

Testing for relationships between landscape and population structure

Dissimilarity matrices of all variables using Euclidian distances were calculated to test for correlations between landscape characteristics (waterway distances and the different measures and types of barriers) and genetic/phenotypic differentiation. Correspondence between matrices was measured using Mantel (Mantel 1967; Legendre & Fortin 1989) and partial Mantel tests (Smouse *et al.* 1986) as implemented in the *ecodist* package in R (Goslee & Urban 2007) with Pearson correlations. *P*-values were obtained with 100,000 permutations.

Eleven different distance matrices were built from comparisons between all possible pairs of populations, and used as independent variables: 1) waterway distance (*geoD*) 2) the total number of barriers (*nbarr*), 3) the total number of artificial barriers (*artbarr*), 4) the total number of natural barriers (*natbarr*), 5) the frequency of barriers (*freqbarr*), 6) different height categories of barriers (*nbarr30*, *nbarr80*, *nbarr150*, *nbarr150+*), 7) the number of weirs (*nweirs*), 8) the number of buildings (*nbuild*), 9) the length of artificial habitat (*arthab*), 10) the length of culverts (*cullength*) and 11) a cumulative measure of all dispersal barriers (*nbarrculart;* see above for calculations of each of these variables). Note that analyses involving *arthab, cullength* and *nbarrculart* only include the 13 tributary populations because there was no geospatial data available on habitat quality in the Aare main river.

Distance matrices used as response variables were 1) genetic differentiation between populations (*genD*) containing pairwise D_{est} estimates, and 2) phenotypic differentiation (*phenDPC1* and *phenDPC2*) containing pairwise P_{ST} estimates based on either PC1 or PC2. The association between phenotypic (*phenD*) and genetic distances (*genD*) between populations was also examined in a Mantel test.

Barriers like waterfalls, weirs or gradation structures with high drops may restrict dispersal only in the upstream direction of waterflow, which can cause complex asymmetric patterns of gene flow among populations (Neville *et al.* 2006a). To take this potential asymmetry in the effect of barriers into account we compared, following Meeuwig *et al*. (2010), the differentiation of populations separated by barriers in only one direction of dispersal (where only one population would have to face barriers when migrating upstream) with the differentiation of populations separated by barriers in both directions (where both populations would have to face barriers migrating upstream). We predicted the degree of differentiation to

be larger in the second group since gene flow is restricted in both directions. Finally, we tested for effects of topographic stream slope on body shape (mean PC2) and genetic diversity (k, H_E) in linear regressions.

Predictions with respect to genetic drift and selection

Analyses were run with the following predictions: 1) If isolation-by-distance was driving population divergence, pairwise D_{est} and P_{ST} estimates should increase with increasing waterway distances. 2) If genetic drift was driving divergence, D_{est} and P_{ST} should increase with an increasing number of dispersal-inhibiting barriers between populations. 3) If diversifying selection across habitats played a more important role in population divergence than genetic drift, geographic distance and the extent of landscape fragmentation would be unlikely to predict spatial patterns in neutral genetic and phenotypic variation. 4) If selection was more important than drift in shaping spatial population structure, phenotypic population differentiation would not be reliably predicted by the extent of neutral genetic divergence. 5) The intensity of stocking per stream correlates negatively with measures of genetic diversity because stocking normally increases the variance in reproductive success between individuals and hence reduces genetic diversity (unless non-native individuals are used for stocking which is not the case in this system).

Assessing the impact of stocking on diversity

In our study area, the Fisheries Inspectorate of the Bern canton controls the stocking of brown trout. Their policy over the last decades stipulates that tributaries can only be stocked with offspring of wild spawners caught from the respective stream, while the Aare main stream can be stocked with offspring of spawners caught in the Aare or tributaries (mainly Aare and Müsche stock was used in the Aare in the last 30 years). As a yearly routine, members of the Fisheries Inspectorate collect wild spawners during the spawning season, use their gametes for generating within-population crosses, and raise the offspring of each stream separately until hatching. Some of these fish are stocked as alevins, but the majority is stocked after six weeks of rearing in tanks or as fingerlings after some weeks in streamlets. Since mortality during this time is mostly not recorded, we used the total number of alevins that were raised in the hatchery since 1981 as a measure of overall stocking intensity per experimental stream. The production of alevins is summarized in Table S1. For the Aare main stream, the numbers of Aare (but not Müsche) individuals released into each of the three Aare sectors were available since 1986. We used these records to generate a proportional distribution key of individuals into sectors (Aare 203: 40.3%; Aare 205: 37.8%; Aare 208: 21.9%). Of the Müsche stock released in the Aare, only the total number of individuals for the entire Aare river was available. We used the distributon key to estimate the stocking effort of Müsche stock into Aare sectors. Individual numbers of Müsche and Aare individuals were then added to obtain the total stocking effort for each Aare sector (Table S1). For Gürbe and Worble, only the total number of individuals per tributary but no sector-specific ('Upper' and 'Lower') numbers were available. We thus assumed an even distribution of the total number of stocked individuals across the two sampling sites.

Assessing population densities

All sampling locations except Müsche were electrofished in mid October 2009 (as described above). All individuals were counted and released thereafter. The tributaries are small enough so that fishing can be considered exhaustive and the total number of brown trout per 100m was taken as a proxy for population density per location. There was no data available on population density in the three Aare sectors. We tested for associations between stocking effort, population density, and genetic diversity using linear regressions.

Results

Genetic diversity

The total number of alleles across populations ranged from 4 to 28 alleles per locus. The mean number of alleles per locus ranged from 4.4 to 19.5 across populations. Allelic richness (k) per population ranged from 7.6 to 10.6. Mean H_E across loci and populations was 0.78 ± 0.02, ranging from 0.73 to 0.81. Mean H_O was 0.74 ± 0.03 ranging from 0.67 to 0.79. No linkage-disequilibrium was found between any pairs of loci, and no deviations from random mating were observed, neither among loci within populations nor among populations within loci. Based on the global test for heterozygote deficit, there were no signs for severe inbreeding (global F_{IS} = 0.046, 95% CI: 0.011-0.08). Overall, genetic population differentiation was moderate (global F_{ST} = 0.022, 95% CI: 0.019-0.025), but as many as 105 (88%) of the 120 possible pairwise population comparisons were significant after Bonferroni correction for multiple testing (Table 2). Some population pairs showed greater genetic dissimilarity than others, yielding a range of discrete F_{ST} estimates from 0.003 to 0.048 (D_{est} estimates ranged from 0.006 to 0.145).

STRUCTURE results suggested that a K value of 6 best describes the number of populations in the 13 tributaries (three-dimensional pie charts in Figure 1 represent the affiliation of each population to the six clusters). The estimated log probability of the data L(K) is large at K of 6, after which there is a break in the slope and variance between replicate runs of K substantially increases (Figure S2a). The maximal value of L(K) returned by the method of Evanno *et al*. (2005) was lower with only 3 clusters (indicated as two-dimensional pie charts in Figure 1, and the highest peak in Figure S2b). Assignment test using discriminant function analysis of principal components (DAPC; Jombart *et al.* 2010) suggested that on average 70% of all individuals could be correctly assigned to their sampling location using multivariate genotypes (for population-specific assignment success see percentages in Table 1).

Phenotypic diversity

We found pronounced differences in body shape morphology among populations (Figure 3). Analysis of multitrait phenotypes produced two major axes of shape variation. PC1 and PC2 explained 16.7% and 10.2 % of total variance in the data set. One-way ANOVAs of 'population' as factor on both PCs were highly significant (PC1: $F_{15,580}$ = 25.2, P < 0.001; PC2: $F_{15,580}$ = 16.2, P < 0.001) indicating population-specific differences in body shape. Discriminant analysis showed that 69% of all individuals (401 out of 581) were correctly assigned to the sampling locations using principal components of body shape (Pillai's Trace = 3.48, P < 0.001). Tukey-Kramer tests showed that 50% (using PC1) and 43% (using PC2) of all pairwise population comparisons were significant. Figure 3 illustrates the morphospace occupied by each population (as examples, the Aare main stem populations, Lower Worble, and Amletenbach are highlighted as green, black and red polgyons, respectively).

Deformation grids in Figure 3 (3 x exaggerated) show body shape changes along the axes. PC1 mainly reflects variation in shape related to sexual dimorphism: On the positive end of PC1, individuals have large heads and long jaws with a rather narrow and straight, almost concave, body shape representing typical male traits. On the negative end, representing the typical female shape, fish have small heads, short jaws and rounded, deep bodies. Only a subset of the phenotyped fish in this study was sexed (n = 48), but sex explained significant amounts of variance along PC1 in this subset (t_{47} = 6.96, P < 0.001), suggesting that indeed most of the variation along PC1 is due to sexual differences in body shape (Figure S3 shows photos of some specimens distributed along PC1). Variation along PC2, however, shows shape changes that are unrelated to sex. The deformation grids demonstrate that individuals located towards the negative extreme of PC2 have small heads with a downward-pointing snout and an overall rather square body shape with a straight back. Towards the positive end of PC2, fish have larger heads with an upwards pointing nose, a terminal mouth, a convex back line, and an overall fusiform shape. The deformation along PC2 predicted from these grids is visualized in Figure S4, where photos of some specimens are plotted and distributed along PC2 (females are shown

on the left, males on the right side of the graph to demonstrate that shape changes along PC2 affect both females and males similarly).

Effects of stream slope on diversity

Variation in body shape was explained by the topographic slope of the streams (mean PC2 within populations: r = 0.58, $P = 0.018$; Figure 4). Populations inhabiting steeper habitats show smaller heads and more downwards pointed mouths whereas populations in shallower habitats have larger heads, terminal mouths and deeper bodies. Genetic diversity was not significantly affected by the slope of the streams (*k* and H_E: $-0.01 < r < 0.01$, $P > 0.73$).

Testing for the effects of habitat fragmentation and isolation-by-distance

Pairwise waterway distance between populations was significantly positively related to their phenotypic differentiation (Mantel tests of *geoD* on *phenDPC2* (P_{ST} estimates based on PC2): $r_M = 0.22$, $P = 0.03$, 95% CI: 0.05-0.42; Figure 5a). Although not significant, a positive trend was also observed for the effect of waterway distance on genetic differentiation (*geoD* on *genD* (D_{est} estimates): $r_M = 0.14$, $P = 0.08$, CI: 0.03-0.28).

Potential dispersal barriers were found every 846m of waterway, on average. The extent of genetic differentiation increased significantly with the number of weirs between populations (*nweirs* on *genD*: $r_M = 0.26$, $P = 0.027$, CI: 0.14-0.40; Figure 5b). None of the other potential barriers (*nbarr, nbarrculart, natbarr, artbarr, nbarr30*, *nbarr80*, *nbarr150*, *nbarr150+*, *freqbarr, nbuild, arthab*) showed an effect on genetic or phenotypic differentiation.

The number of dispersal barriers is often linked to the total waterway distance between populations and can thus confound the effects of isolation-by-distance and habitat fragmentation. Indeed, the number of weirs increased with increasing geographic distance between populations (*nweirs* on *geoD*: $r_M = 0.51$, $P < 0.001$, CI: 0.40-0.62, Figure S5). We thus investigated the relationship between the three distance matrices (*nweirs*, *geoD*, *genD*). A partial Mantel test on the impact of weirs after removing isolation-by-distance effects was close to significance (*nweir + geoD* on *genD*: $r_M = 0.22$, $P = 0.06$, CI: 0.09-0.36). Partial Mantel test on isolation-by-distance after removing weir effects did not yield significance (*geoD + nweir* on *genD*: $r_M = 0.01$, $P = 0.89$, CI: -0.07-0.11).

Individuals collected from the three locations along the main stream may be less likely to represent biological populations than individuals collected in the tributaries since spatial connectivity in the main stream is probably higher, potentially blurring isolation-by-distance between locations. At the same time, divergence between tributary populations may be caused by the dendritic habitat structure itself (Carlsson *et al.* 1999; Kanno *et al.* 2011). We thus ran analyses excluding the three Aare main stem populations 208, 205, and 203. Tested on tributary populations only, divergence increased both genetically (*geoD* on *genD*: $r_M = 0.26$, $P = 0.018$, CI: 0.13-0.47; Figure 5c) and phenotypically (*geoD* on *phenDPC2*: $r_M = 0.29$, $P = 0.023$, CI: 0.014-0.58) with waterway distance. The effect of weirs was again close to significance (*nweirs* on *genD*: $R = 0.25$, $P = 0.08$, CI: 0.12-0.43).

The longer the culverts were between populations, the more different populations were phenotypically (*cullength* on *phenDPC2*: $r_M = 0.41$, $P = 0.006$, CI: 0.24-0.57; note that this result includes only tributary populations as no information on habitat quality was available for the Aare main stream).

Using the entire data set, including the Aare main stream populations, the extent of eco-morphological differentiation between populations was not associated with their genetic differentiation (*genD* on *phenDPC1:* $r_M = -0.04$, $P = 0.78$, CI: -0.13-0.15; *genD* on *phenDPC2:* $r_M = -0.08$, $P = 0.37$, CI: -0.16-0.03; Figure 5d).

ANOVA testing for dispersal asymmetry (comparing the differentiation of populations separated by barriers in only one direction of dispersal with differentiation of populations separated by barriers in both dispersal directions) did not show significant differences between

the two groups ($F_{1,155} = 0.11$, $P = 0.74$), suggesting that dispersal asymmetry does not play a prominent role in shaping population structure in this system.

In summary, genetic population divergence was positively related to the extent of fragmentation by weirs. When tested among tributary populations, genetic divergence also increased with waterway distance. In addition, the longer the waterway distances between populations, and the more culverts between them, the higher their phenotypic divergence. However, effects of waterway distance could not be conclusively disentangled from effects of fragmentation by weirs.

Impact of population density and stocking

Genetic diversity increased with increasing population density (k: r = 0.69, n = 12, $P = 0.013$; H_E: r = 0.79, $P = 0.002$; Figure 6a, excluding Müsche and Aare). Stocking intensity did not significantly correlate with population density (r = 0.23, n = 12, $P = 0.46$) or with the observed genetic diversity in the tributaries, but there was a positive trend (k: r = 0.52, n = 13, $P = 0.068$; H_E: r = 0.46, $P = 0.11$; Figure 6b, the Aare populations were excluded here due to the mixed origin of their stock). F_{IS} was not predicted by population density (r = -0.28, n = 12, $P = 0.39$), stocking intensity (r = -0.12, n = 13, $P = 0.69$), or the total number of females used for the stocking program (r = 0.0, n=13, $P = 0.98$).

Discussion

We investigated the genetic and phenotypic diversity and population divergence of brown trout inhabiting a 40 km long stretch of the Aare river (Switzerland) and its tributaries. We tested whether incorporating landscape features along dispersal corridors better correlates with patterns of population differentiation than a pure isolation-by-distance model.

Genetic and phenotypic diversity

We found overall high genetic diversity with no signs of inbreeding, and only slight variation between populations in observed heterozygosity and allelic richness. Global population differentiation was moderate (global $F_{ST} = 0.022$) as expected for a network of salmonid populations within the same catchment (Carlsson & Nilsson 2000; Jensen *et al.* 2005a; Heggenes & Roed 2006; Griffiths *et al.* 2009; Lehtonen *et al.* 2009; Hansen *et al.* 2010; Kanno *et al.* 2011). Populations differed considerably both genetically and phenotypically. Eighty-eight percent of all pairwise F_{ST} comparisons, and 50% (along PC1) and 43% (along PC2) of all comparisons in body shape were significant. The high success rates of assignment tests using genotypic (70%) and phenotypic data (69%) confirm the distinctiveness of populations. The high rates of significant pairwise F_{ST} comparisons found here (despite low global F_{ST} values) seem typical for resident salmonids (100% of all pairwise population comparisons were significant in Carlsson & Nilsson 2001; 100% in Jensen *et al.* 2005b; 86% in Heggenes & Roed 2006; 76% in Griffiths *et al.* 2009; 34% in Junge *et al.* 2011).

These data suggest that brown trout in the Aare system diverged genetically and phenotypically on a small geographic scale (pairwise waterway distances between populations ranged from 2 to 40km), in agreement with several studies on resident salmonid populations that found geographical structuring on a similarly small spatial scale (Estoup *et al.* 1998; Carlsson & Nilsson 2000; Barson *et al.* 2009; Griffiths *et al.* 2009; Kanno *et al.* 2011).

Keller *et al*. (2011) recently presented evidence for neutral and adaptive divergence to altitude in brown trout in Switzerland. Their sampling region encompassed a geographically larger area including three central European drainage systems (Rhone, Rhine and Po), but also included individuals from two tributaries sampled in this study (Kiese and Rotache). Our study takes a magnifying glass to Keller *et al*'s results, revealing that considerable genetic and phenotypic variation occurs even on a much smaller geographic dimension, within the same area, demonstrating that even small tributary populations, which may be low in productivity,

can significantly contribute to the overall genetic diversity of a region.

The microgeographic structuring found here speaks for spatially restricted populations that do not frequently disperse long distances. Whatever may be the reason (e.g. ecological habitat preferences or physical barriers), constrained dispersal ranges and restricted gene flow make these populations vulnerable to anthropogenic change. Even though an increasing number of studies demonstrates that salmonids can respond to environmental change within 6-30 generations (Haugen & Vøllestad 2000; Hendry *et al.* 2000; Quinn *et al.* 2001; Unwin *et al.* 2003; Kinnison *et al.* 2008; Kavanagh *et al.* 2010; Fraser *et al.* 2011), the speed of adaptive evolution depends on the interplay between gene flow, genetic drift, the strength of selection and standing genetic variation (Adkison 1995; Hansen *et al.* 2002; Hansen *et al.* 2007; Hendry *et al.* 2007). In order for populations to be able to adapt to changing environmental conditions, their genetic diversity must be maintained. In this context, stocking is a rather controversial practice (Araki *et al*. 2007; Fraser 2008; Eldridge *et al*. 2009). Domestication selection in hatcheries, or increased genetic drift caused by stocking, can potentially lead to the loss of local adaptation (Hansen *et al.* 2009; Muhlfeld *et al.* 2009), and hatchery fish can have lower longterm fitness in the wild (Poteaux *et al.* 1998; Hansen *et al.* 2001; Ruzzante *et al.* 2001). On the other hand stocking may, at times, save populations through ecological crises (e.g. when natural spawning places are temporally lost).

The river Aare and most of its tributaries have been systematically stocked since decades. Stocking typically increases the variance in reproductive success between individuals within a population, which is usually expected to lead to an erosion of genetic diversity (unless non-native individuals are used for stocking which is not the case in this system) (Laikre *et al.* 2010). Contrary to these expectations, we found positive correlations between stocking intensity and genetic diversity that were close to statistical significance. It is likely that this pattern is caused by the fact that stocking is more intense in rivers with larger populations, and because larger census size increases genetic diversity (supported here by the finding that diversity increases with population density). These results leads us to conclude that there is no significant stocking-induced genetic drift, and that stocking is not likely to be the driver of population divergence here.

Reliable long-term data on stocking effort are notoriously difficult to obtain and it is quite unusual for a central European river system like the Aare to have such detailed data on hatchery-raised breeding stock at hand. However, our conclusions are based on comparably rough measures of stocking intensities and widely varying numbers across the last 30 years. Proportional estimates of the number of wild versus stocked fish, for instance, would be useful but were unavailable. In addition, little is known about the effectiveness of the stocking program in this region. It should be noted that in the Aare system, any cross-stocking between tributaries, which could erode genotypic and phenotypic diversity over time (Largiadèr & Scholl 1995), is prohibited. Any steward leasing a brook or streamlet, signs an agreement that their entire stock is obtained from the same state-run hatchery and this regulation has been operative for decades (C. Küng, Head of Fisheries, Berne canton, pers. comm.). The only exception is the Aare main stem, which is regularly stocked with fish from the Müsche (because high numbers of spawners are caught in the Müsche while the number of spawners obtained in the Aare has been continuously declining since the 1980s). However, increased similarity of the genetic composition of these two populations, potentially caused by artificially enhanced gene flow through stocking, was not identified here.

Divergence mechanisms

We could not disentangle the effects of isolation-by-distance and habitat fragmentation on divergence (pairwise waterway distances and the number of weirs between populations covaried). We found that one type of barrier, the number of weirs between populations, may contribute to genetic differentiation. In agreement with this, weirs (Meldgaard *et al.* 2003) and

other geomorphological features (e.g. Ferguson 1989; Moran *et al.* 1995; Bouza *et al.* 1999; Ruzzante *et al.* 2001; Heggenes & Roed 2006) have been identified to physically reduce gene flow between salmonid populations. At the same time, we found that waterway distance predicted genetic population differentiation, at least among tributary populations. It has been suggested that the network structure of tributaries can play a role in population divergence (Carlsson *et al.* 1999; Kanno *et al.* 2011), and movement between branch populations may be limited due to ecological factors (habitat preferences and philopatry). With regard to phenotypic differentiation, we found that the total length of culverts (subterranean canalization) between sampling sites correlated with divergence in overall body shape. In summary, both artificially induced genetic drift due to habitat fragmentation and natural effects of isolation-by-distance may affect population divergence in this system. Because the effects of geographical distance and habitat fragmentation are confounded here and because of its stocking history, the Aare river may not be the ideal system to investigate the degrees with which drift or selection contribute to population divergence.

Interestingly, the majority of studies on salmonid networks do not reveal isolation-by-distance effects (Ryman 1983; Crozier & Ferguson 1986; Ferguson 1989; Moran *et al.* 1995; Hansen & Loeschcke 1996; Bouza *et al.* 1999; Carlsson & Nilsson 2001; Castric *et al.* 2001; Ruzzante *et al.* 2001; Meldgaard *et al.* 2003; Heggenes & Roed 2006), with a few exceptions (Estoup *et al.* 1998; Carlsson & Nilsson 2000; Griffiths *et al.* 2009; Lehtonen *et al.* 2009). A possible explanation for this is that isolation-by-distance can become obscured by analytical artefacts, i.e. insufficient resolution of molecular markers, unsuitable statistical approaches (see for review Jaquiéry *et al.* 2011), and sampling regimes not fine-scaled enough with respect to the geographical locations of the populations and their dispersal abilities (Dungan *et al.* 2002; Anderson *et al.* 2010; Cushman & Landguth 2010). We accounted for these potential pitfalls here: We applied a fine-scaled geographic sampling regime and used mostly large sample sizes. The number of microsatellites used here (n = 11), the fact that they were in linkage equilibrium and their level of allelic information content is usually considered sufficient to resolve population structure (e.g. Gomez-Uchida *et al.* 2009; Griffiths *et al.* 2009; Taylor *et al.* 2011). Multivariate genotypes were assigned to their source populations with 70% success rate and DAPC ensured that the variables entering the models were uncorrelated. Lastly, Mantel tests are conservative with regard to spatial autocorrelation and false positives generated by the non-independence of the pairwise data (Epperson 2010).

Functional interpretation of variation body shape

Ecological selection can lead to local adaptation or phenotypically plastic responses, allowing individuals to have higher relative fitness in their native habitat than non-natives (Dobzhansky, 1968; Kawecki & Ebert, 2004). As a consequence, gene flow between populations may become reduced due to selection against immigrants (Schluter 2000; Hendry 2001; Hendry 2004; Nosil 2004; Rundle & Nosil 2005) and lead to divergence among populations, even without physical isolation (Piertney *et al.* 2001; Koskinen *et al.* 2002; Irwin *et al.* 2005). It is possible that the morphological differentiation we found between populations reflects some level of adaptive divergence or plastic phenotype-habitat matching, instead of being the mere byproduct of isolation-by-distance and habitat fragmentation.

Our assessment of body shape revealed some interesting morphological differences, allowing for some speculation on the ecology of populations. While variation along PC1 was mainly explained by sexually dimorphic traits, shape changes along PC2 point to (genetic or plastic) adaptations of trophic and swimming structures, affecting both males and females similarly. Populations inhabiting tributaries with a steeper slope (e.g. Zulg) seem to have overall shallower bodies, smaller heads and downward pointing mouths. These may well be adaptations to a life style in shallow and fast flowing water, where feeding on food items attached to the stream bed (like caddisfly larvae) and a body shape that allows hiding behind rocks (e.g. like a

bottom dwelling sculpin) is advantageous. Populations in slow flowing, wider and deeper streams (e.g. Giesse Münsingen) have large heads and jaws with terminal or upward pointing mouths, which may be adaptations to a more predatory life style, e.g. allowing them to hunt for small fish in open water.

Variation in overall body shape is one of the best-known morphological responses in salmonids to flow regime (Riddell & Leggett 1981; Taylor 1991; Hendry *et al.* 2000; Pakkasmaa & Piironen 2000; Langerhans 2008; Paez *et al.* 2008) and has been demonstrated to be heritable several times independently (Hard *et al.* 1999; Hendry 2001; Boulding *et al.* 2008; Varian & Nichols 2010). Fusiform shapes (such as those on the positive end of PC2) are generally thought to improve sustained swimming and foraging in open waters (Taylor & Foote 1991), which is consistent with the potential predatory function of the large head and jaws we found here. Also the size of the water body has been shown to affect the body shape of salmonids. Larger streams (and lakes) are typically inhabited by more robust fish with larger heads, bodies and fins (Beacham & Murray 1987). With respect to head morphology, studies in other species of fish have predicted (Wainwright & Richard 1995) and confirmed (Nicieza 1995; Blackie *et al.* 2003) that variation in head shape can reflect adaptations to different dietary sources.

Although our results do not establish a causal relationship between the ecological conditions these populations encounter and their body shape, the streams in this study show pronounced differences not only in slope, but also in other physical parameters (e.g. rate of discharge, flow velocity, depth, width, substrate, degree of shading, temperature; data not shown here). It thus seems that there is great potential in this system for phenotypic variation to be generated by local adaptation and/or plasticity. In summary, the differences in body shape found in different tributary populations may be the result of population divergence mediated, at least to a degree, by diversifying ecological selection for (genetic or plastic) adaptation to flow velocity and dietary compounds.

Phenotypic divergence without genetic divergence

Phenotypic and genetic differentiation were not associated in our study. This may be because traits under strong diversifying selection can display fine-scale spatial variation without significant differentiation at neutral loci, which would be in accordance with the above finding that trophic and swimming structures varied with ecological habitat heterogeneity, whereas genetic differentiation did not. It has been demonstrated in salmonids (Hendry *et al.* 2000; Junge *et al.* 2011) and other species (Stockwell *et al.* 2003; Hairston *et al.* 2005) that local adaptation can eventually overcome the homogenizing effects of gene flow, but that the phenotypic response to environmental change generally exceeds the genetic response in salmonids (Koskinen *et al.* 2002; Rogers *et al.* 2002; McClelland & Naish 2007; Jensen *et al.* 2008; Kinnison *et al.* 2008) and other species (e.g. Trussell & Smith 2000; Both & Visser 2001; Reale *et al.* 2003).

Spatially structured, phenotypic diversity without significant genetic divergence can also be the result of phenotypic plasticity. A recent review (Hutchings 2011) on salmonids summarizes evidence that population differences in reaction norms can reflect adaptive responses to local environments, and that phenotypic plasticity itself is heritable. Hendry *et al.* (2008) and others (e.g. Charmantier *et al.* 2008) argue that human-induced environmental change can be bridged by the plasticity of wild populations. The plastic response places the population closer to a fitness peak, which may aid population persistence by weakening selection (Price *et al.* 2003; Chevin & Lande 2010; Luquet *et al.* 2011). It has been shown that phenotypic plasticity can 'rescue' populations from extinction by increasing survival in fragmented landscapes (Letcher *et al.* 2007). As a consequence, the genetic response of traits under selection may be slowed down by plasticity (Price *et al.* 2003) explaining the lack of genetic population differentiation. Our results are consistent with this slow-down effect, but

more detailed analyses are needed to determine if any of these mechanisms are operational.

Conclusions

Substantial genetic and phenotypic variation was found among brown trout populations within a geographically small area (within 40km). Spatial substructure in genetic and phenotypic diversity was correlated to fragmentation by weirs and, in the case of tributary populations, to isolation-by-distance effects. Phenotypic divergence was also related to the length of poor habitat between populations. Eco-morphological differences in trophic and swimming structures among populations could be largely explained by the topographic slope of the streams, indicative of differences in trout habitat. These differences speak for the presence of local adaption or phenotypic plasticity to flow velocity, stream size or dietary resources. The interdisciplinary approach applied in this study, integrating population genetics, multivariate phenotype analysis, and landscape analysis, can serve to uncover cryptic genetic and phenotypic diversity. A sound understanding of the degree and scale of population divergence may be crucial for management and conservation.

Acknowledgements

We dedicate this paper to the memory of H. Walther who generously provided much advice and assistance in this and other collaborations with the Reutigen Hatchery. We thank E. Baumgartner, K. Bettge, A. Bréchon, P. Büsser, E. Clark, R. Droz, A. Escher, W. Grossenbacher, U. Gutmann, J. Hanimann, J. Knörr, L. Kocjancic-Curty, C. Kropf, P. Landolf, S. Nusslé, M. Pompini, A. Ross-Gillespie, F. Russier, M. Schmid, and K. von Wattenwyl for support and assistance in the field and in the laboratory. We thank C. Küng from the Fisheries Inspectorate Bern for permissions, and C. Küng, U. Gutmann, and other staff of the Reutigen Hatchery for sharing information on stocking programs since 1981. We are indebted to J.P. Danko, M. dos Santos, J. El-Assad, A. Espindola, J. Goudet, S. Mariani, S. Nusslé, C. Tanner, E. Taylor, K.A. Young, and two anonymous reviewers for critical review and various helpful comments. This study complied with the relevant ethical regulations imposed by the university, canton, and country in which it was carried out. The Swiss National Science Foundation, the Bern canton, and the *Maison de la Rivière* provided funding.

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Table Legends

Table 1. Location, coordinates, number of individuals sampled, and neutral genetic variation of the study populations determined from eleven microsatellite loci. None of the F_{IS} values differed significantly from zero, i.e. there were no signs for heterozygote deficits in any population. Percentages in the last column represent population-specific assignment success in discriminant function analysis of principal components (DAPC) using multivariate genotypes.

Table 2: Estimates of pairwise population differentiation. F_{ST} values shown above diagonal, D_{est} values below diagonal. Asterisks indicate significance at the 5% nominal level after Bonferroni correction (non-significant comparisons in italics). 105 out of 120 pairwise population comparisons were significant.

Figure 1: Map of the study area (Aare catchment between Lake Thun and the city of Bern, Switzerland) with collection sites indicated as white circles. Dashed arrow indicates direction of water flow. The box in the upper-right corner indicates the location of the catchment in Switzerland. Surface elevation is taken from the *swissALTI3D Reliefschattierung* (Bundesamt für Landestopografie; map.geo.admin.ch). Three-dimensional pie charts indicate cluster affiliation according to results from STRUCTURE (L(*K*) = 6). Two-dimensional pie charts indicate cluster affiliation according to the method by Evanno *et al*. (2005) ($\Delta K = 3$).

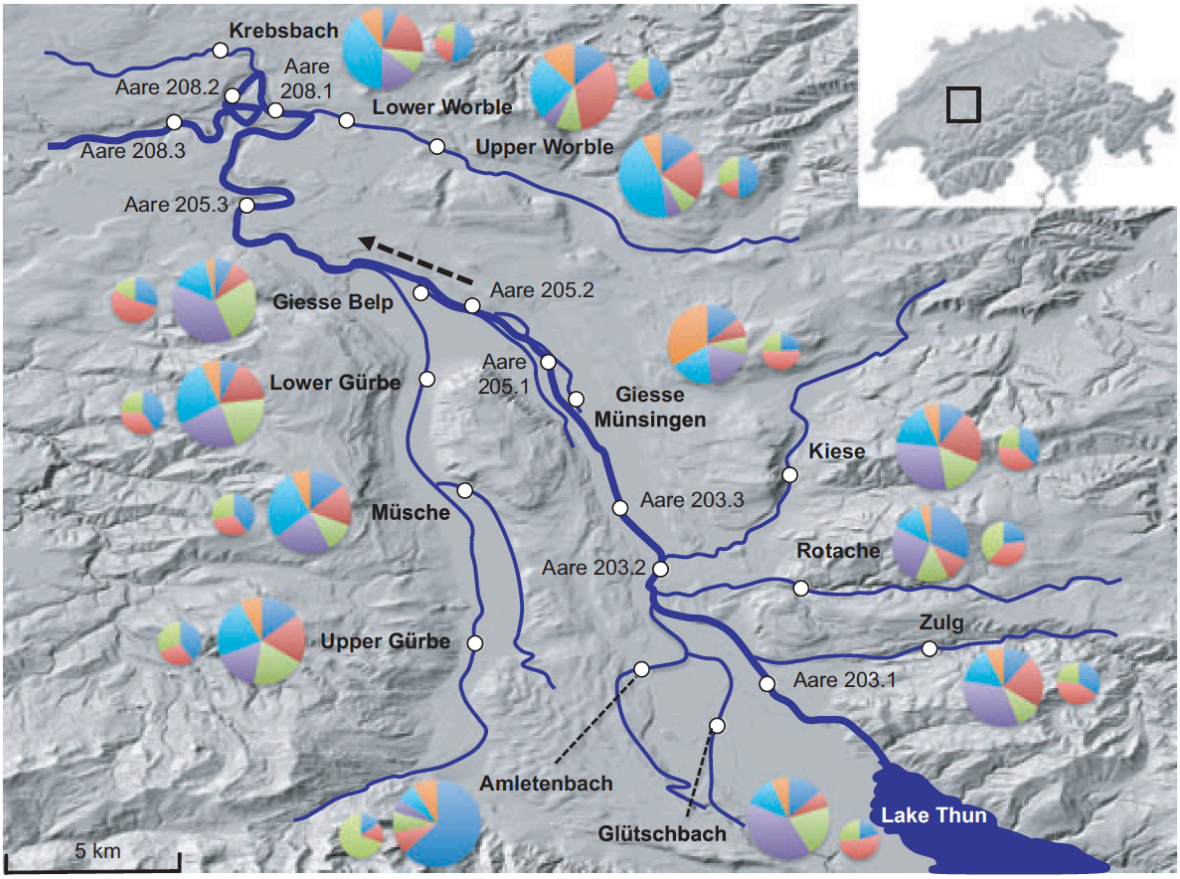


Figure 2: Position of the 13 landmarks used to describe body shape variation. (1) anterior tip of snout, (2) centre of the eye, (3) posterior end of neurocranium, (4) origin of dorsal fin, (5) origin of adipose fin, (6) anterior attachment of dorsal membrane of the caudal fin, (7) base of middle caudal rays (8) anterior attachment of ventral membrane of the caudal fin, (9) origin of anal fin, (10) origin of pelvic fin, (11) origin of pectoral fin, (12) posterior end of maxillary (13) posterior end of brachiae (modified from Varian & Nichols 2010).

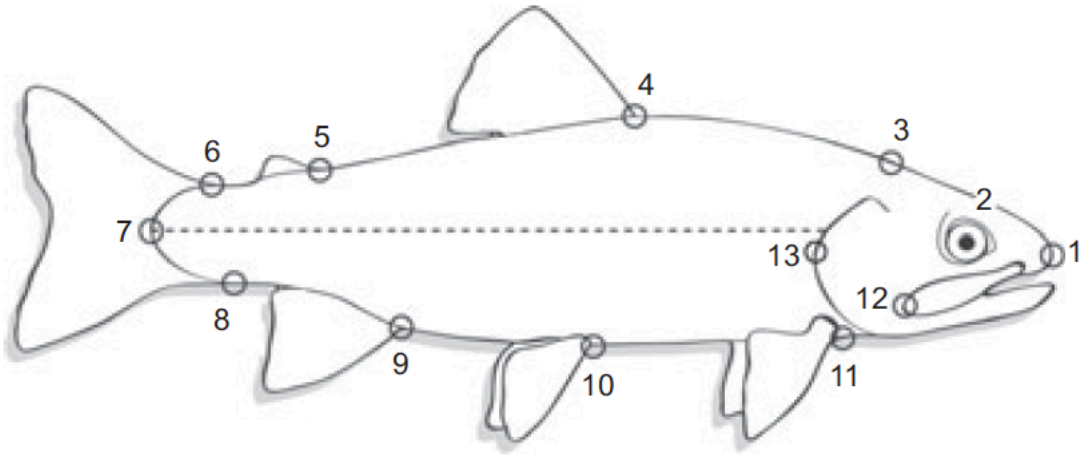


Figure 3: Results of principal component analysis using geometric morphometric data to test for body shape differences among populations. Graph shows variation along the first two major axes of shape variation (PC1 and PC2, % of total variance in brackets). Every data point is an individual. To exemplify, three populations are outlined with polygons for visualization. The green polygon shows the morphospace of the three Aare main stem populations combined. Body shape deformation grids (3 x exaggeration) at the extremes of each axis visualize morphometric change along the two major shape trajectories. Potential interpretations of shape changes are given at the end of each axis.

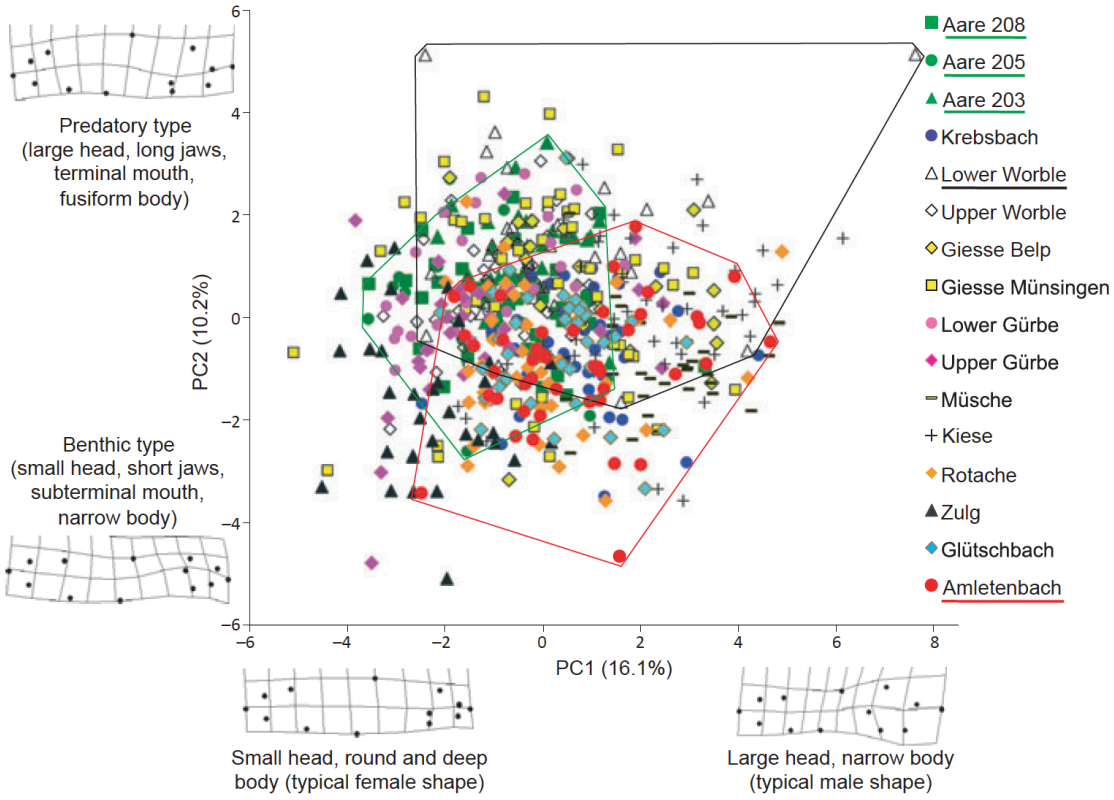


Figure 4: The relationship between topographic stream slope and body shape (mean PC2 within populations).

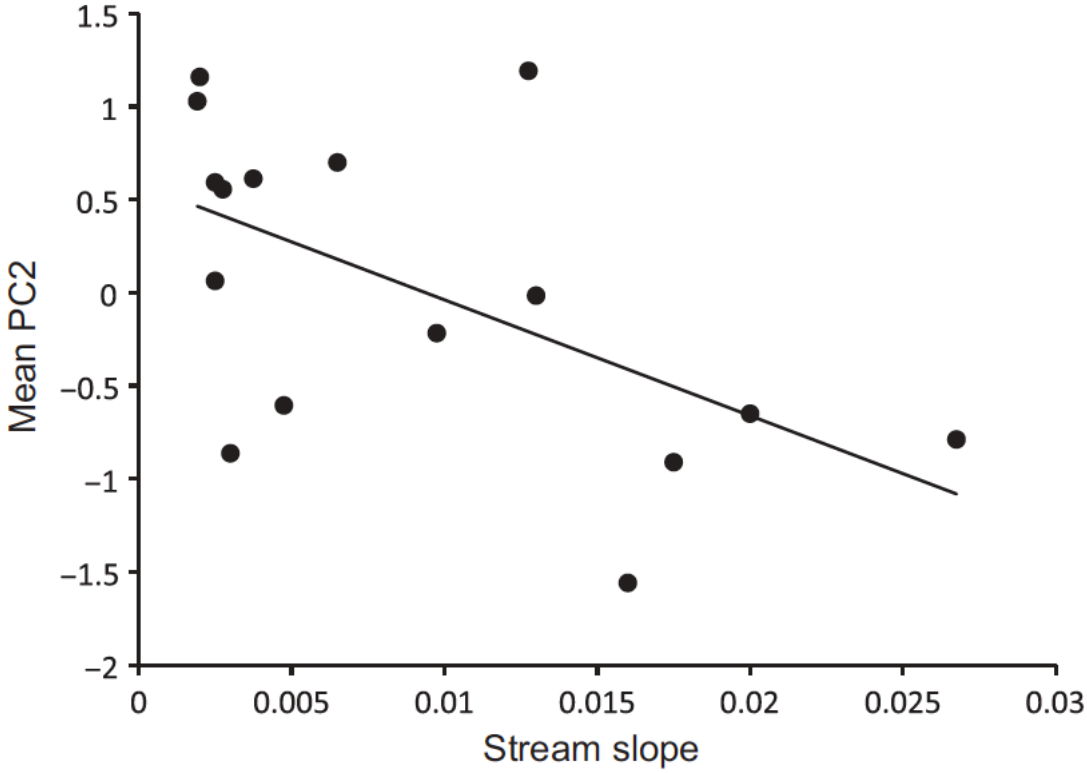


Figure 5: Potential predictors of genetic and phenotypic population differentiation. Graphs show linear regression on untransformed data with each data point representing a pairwise population comparison (Mantel statistics given in the text account for spatial autocorrelation). a) The extent of phenotypic differentiation (P_{ST} based on PC2) between populations was significantly positively correlated (in Mantel tests) to waterway distance (km). b) The extent of genetic differentiation (D_{est}) was significantly positively correlated to the number of weirs. c) Genetic differentiation was significantly positively correlated to waterway distance. This graph shows a subset of data including tributary populations only. d) Phenotypic (P_{ST} based on PC2) and genetic (D_{est}) differentiation were not correlated.

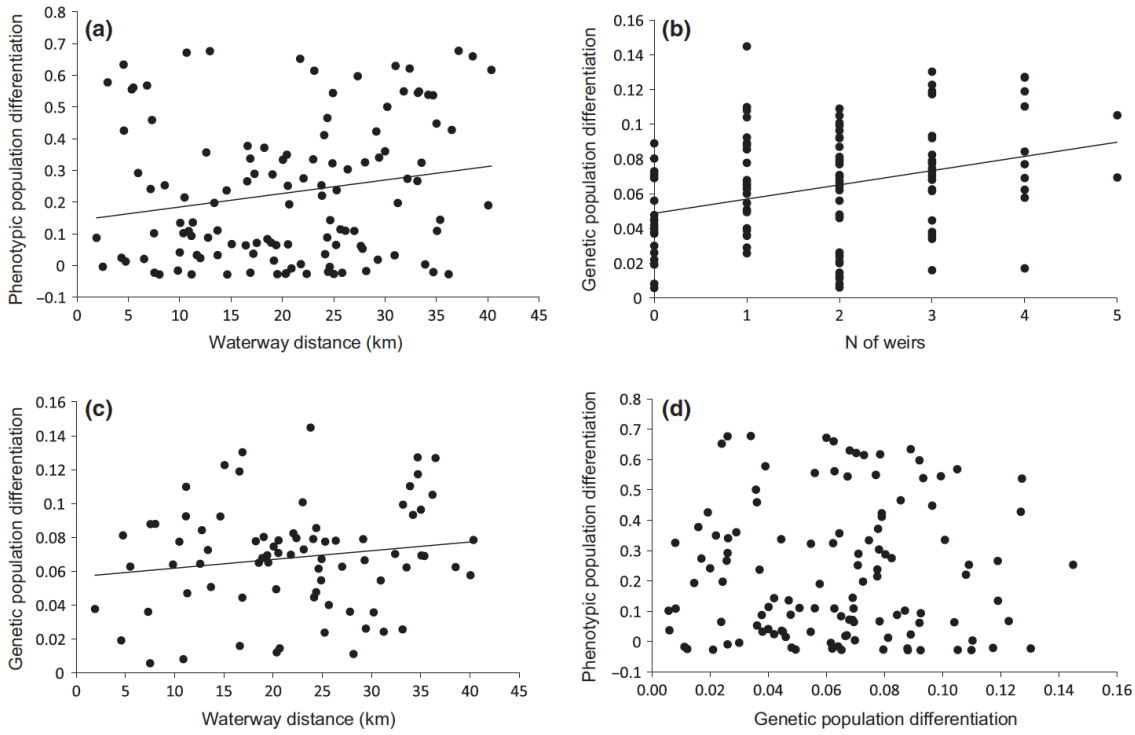
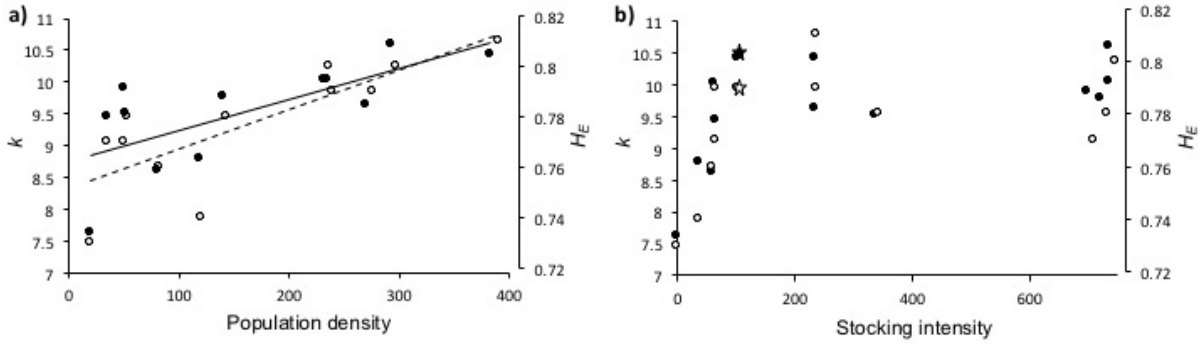


Figure 6: The relationship between measures of genetic diversity (*k*: filled symbols, solid line; H_E = open symbols, hatched line) and a) population density; and b) between measures of genetic diversity and stocking intensity (total number of alevins produced in the hatchery over the last 30 years; in thousand). Sample sizes vary because population density was not estimated for Müsche (indicated with stars). However, exceptionally high numbers of spawners can regularly be caught from this comparatively small stream, further supporting the correlations in panel a. Lines represent significant regressions. See text for statistics.



Supplementary Material

Table S1: Summary of the hatchery program for brown trout within the study area since 1981. While in some years small proportions were stocked as alevins or 1+ fish, the large majority were raised for some weeks in the hatchery or in small streamlets before released into the stream of origin, with the exception that the river Aare was regularly stocked also with fish originating from other streams of the catchment area (mainly Müsche).

River/stream	n years [1]	n females per year [2]	n alevins per year [2,3]	Total n alevins in 30 years [3]	Total n of alevins used for stocking[3,4]
Aare 203					1923
Aare 205	25	141.2 (6-476)	119.2 (4.1-341.7)	2,980	1803
Aare 208					1045
Gürbe Upper	23	110.7 (18-270)	63.8 (8.6-140.4)	1,468	734
Gürbe Lower					734
Worble Upper	14	75.7 (17-189)	33.4 (6.6-73.5)	468	234
Worble Lower					234
Amletenbach	3	64.0 (41-77)	19.8 (13.6-26.9)	59	59
Kiese	22	103.3 (6-266)	32.8 (2.8-66.5)	721	721
Giesse Belp	0	0	0	0	0
Giesse Münsingen	18	54.7 (15-99)	38.7 (5.6-76.1)	697	697
Glütschbach	3	47 (18-85)	11.5 (6.1-16.9)	35	35
Krebsbach	4	46.0 (37-65)	15.7 (10.9-20.4)	63	63
Rotache	14	80 (30-135)	24.1 (7.4-41.1)	337	337
Zulg	5	46.2 (15-88)	12.7 (3.4-22.0)	64	64
Müsche	17	284.1 (170-415)	111.3 (49.4-179.9)	1,894	102

[1] number of years that spawners were collected from the respective stream between winter 1981/82 and 2010/11

[2] mean and range

[3] in thousands

[4] in the respective stream or river sector; total n of alevins adjusted as explained in Methods

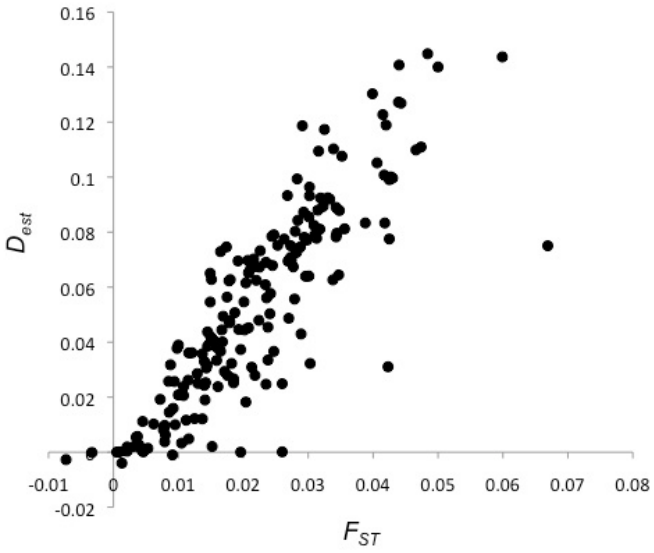


Figure S1: Correspondence between F_{ST} and D_{est} estimates.

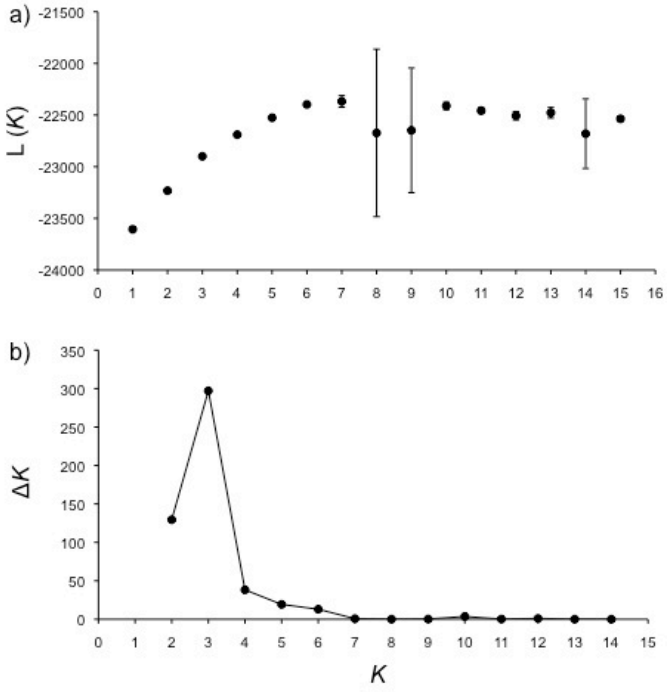


Figure S2: Graphical analyses to infer the most likely *K* value. a) Mean L(*K*) (± sd) as a function of the number of clusters (*K*) across 6 runs obtained with STRUCTURE. The 'break in slope' at K=6 represents the most likely n of K. b) After applying the method described in Evanno et al. (2005), the highest peak represents the most likely *K* value (= 3). Δ*K* was calculated as $\Delta K = m|L''(K)|/s[L(K)]$.

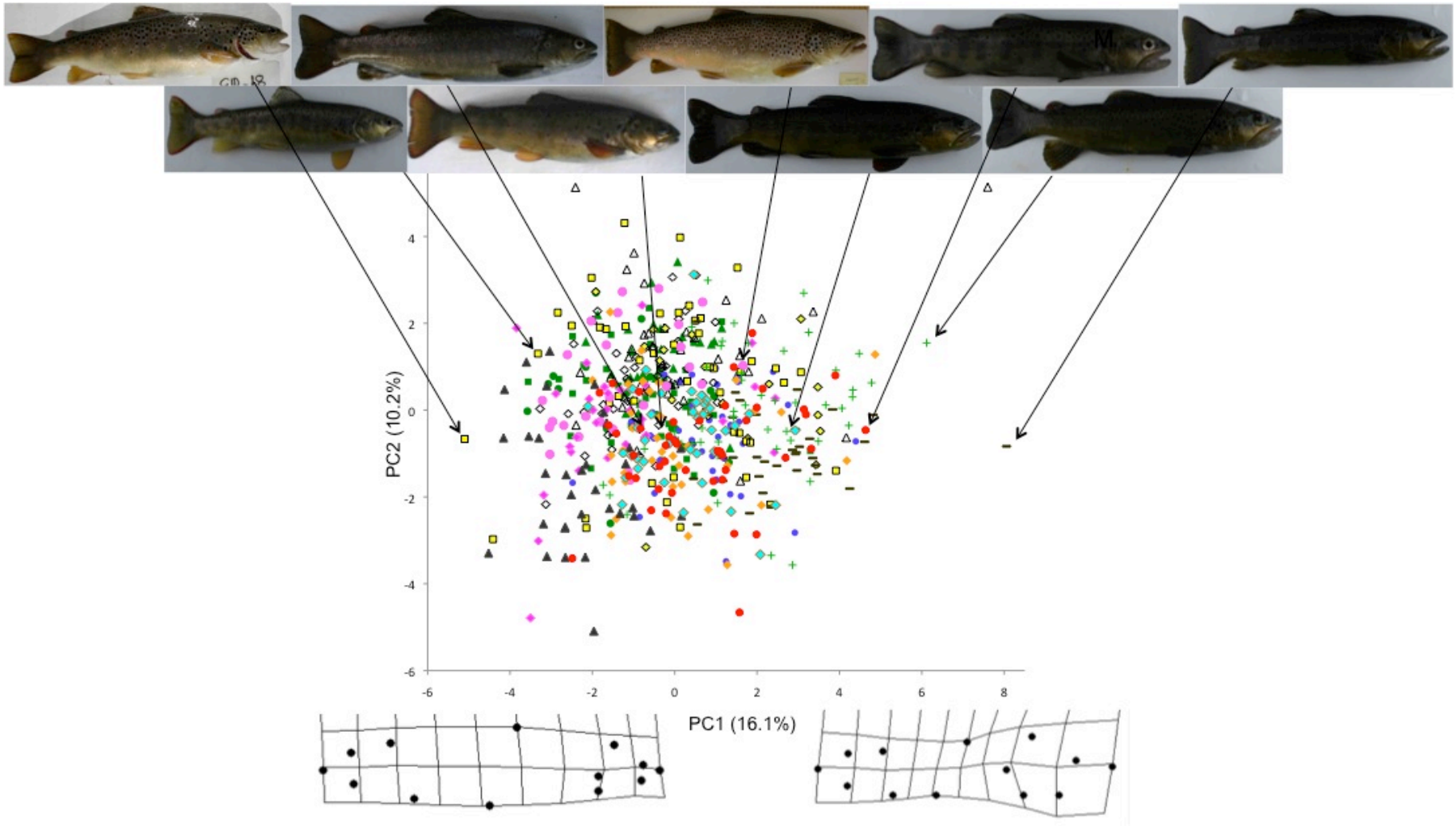


Figure S3: Same as Figure 3 with photos of example specimens plotted along principal component 1 (x- axis). Deformation grids represent shape at the negative and positive extremes of PC1.

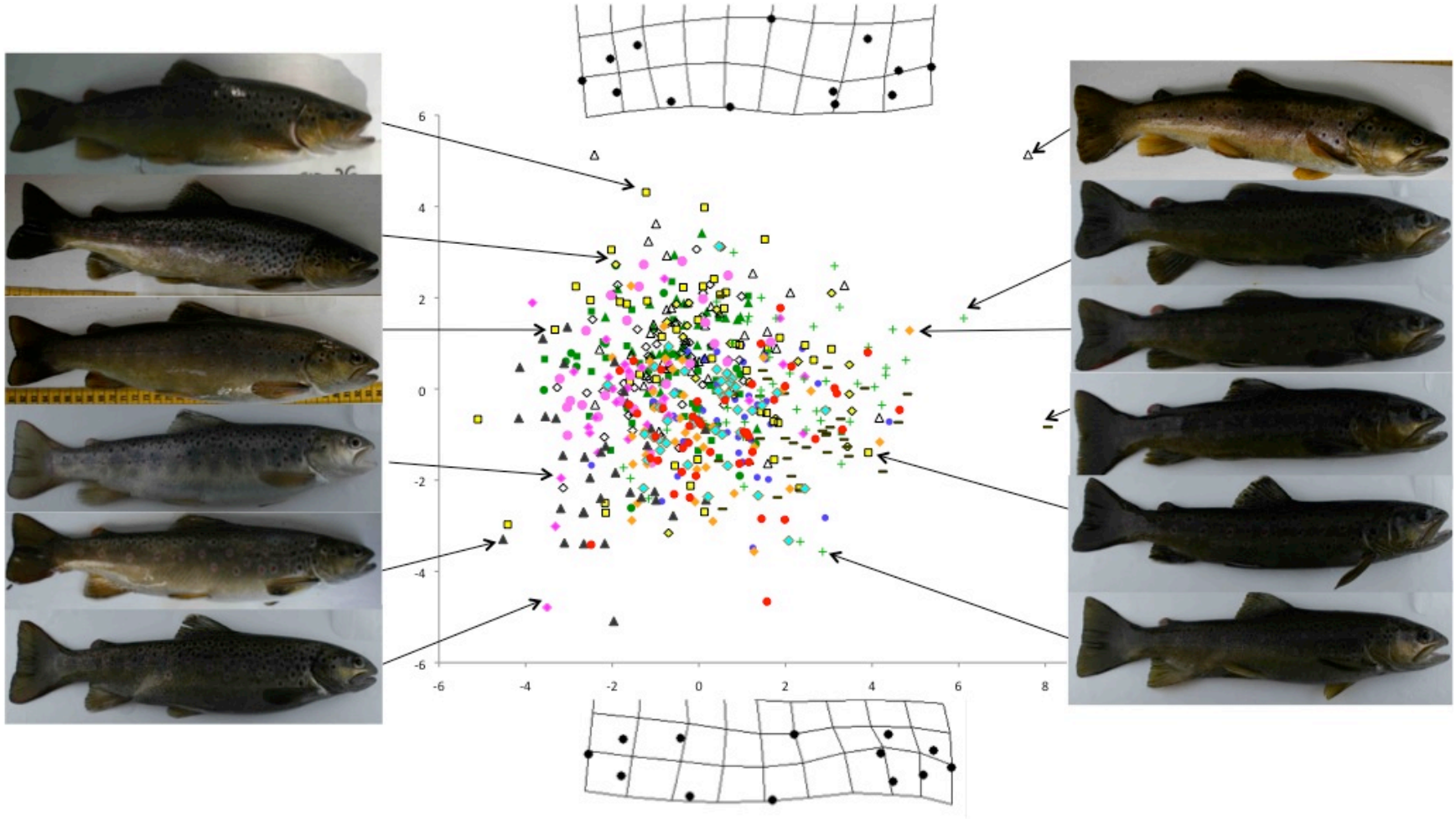


Figure S4: Same as Figure 3 with photos of example specimens plotted along principal component 2 (y- axis). Photos on the left show females, photos on the right show males. Deformation grids represent shape at the negative and positive extremes of PC2.

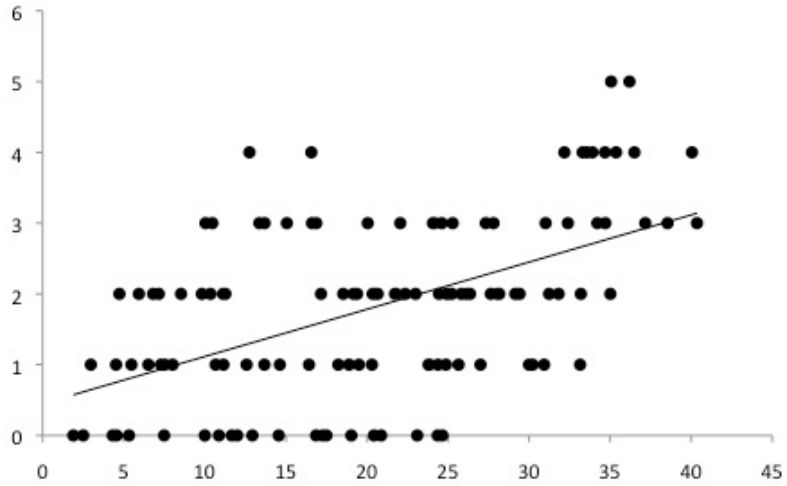


Figure S5: The number of weirs (*nweirs*) was positively related to waterway distance (*geoD*) between pairs of populations (Mantel test: $R = 0.051$, $P < 0.001$). Every data point represents a pairwise population comparison.