Rapid adaptation through genomic and epigenomic responses following translocations in an endangered salmonid

Crotti, M.¹, Yohannes, E.³, Winfield, I.J.⁴, Lyle, A.A.², Adams, C.E.¹²*, Elmer, K.R.¹*

¹ Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
² Scottish Centre for Ecology and the Natural Environment, University of Glasgow, Rowardennan, G63 0AW, UK
³ Limnological Institute, University of Konstanz, Konstanz, 78464, Germany.
⁴ Lake Ecosystems Group, UK Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK

*Authors for correspondence

Marco Crotti: m.crotti.1@research.gla.ac.uk
Elizabeth Yohannes: elizabeth.yohannes@uni-konstanz.de
Ian Winfield: ianjwinfield@icloud.com
Alex Lyle: alexlyle03@yahoo.co.uk
Colin Adams: Colin.Adams@glasgow.ac.uk
Kathryn Elmer: Kathryn.Elmer@glasgow.ac.uk

Running title: Translocations for whitefish conservation
Abstract

Identifying the molecular mechanisms facilitating adaptation to new environments is a key question in evolutionary biology, especially in the face of current rapid and human-induced changes. Translocations have become an important tool for species conservation, but the attendant small population sizes and new ecological pressures might affect phenotypic and genotypic variation and trajectories dramatically and in unknown ways. In Scotland, the European whitefish (*Coregonus lavaretus*) is native to only two lakes and vulnerable to extirpation. Six new refuge populations were established over the last 30 years as a conservation measure. In this study we examined whether there is a predictable ecological and evolutionary response of these fishes to translocation. We found eco-morphological differences, as functional traits relating to body shape differed between source and refuge populations. Isotopic analyses suggested some ecological release, with the diets more diverse in refuge populations than in source populations. Analyses of up to 9,117 genome-mapped SNPs showed that refuge populations had reduced genetic diversity and elevated inbreeding and relatedness relative to source populations, though genomic differentiation was low ($F_{ST} = 0.002 – 0.030$). We identified 14 genomic SNPs that showed shared signals of a selective response to translocations, including some located near or within genes involved in the immune system, nervous system, and hepatic functions. Analysis of up to 120,897 epigenomic loci identified a component of consistent differential methylation between source and refuge populations. We found that epigenomic and genomic variation were associated with morphological variation, but we were not able to infer an effect of translocation age because the patterns were also linked with the methodology of the translocations. These results show that conservation-driven translocations affect evolutionary potential by impacting both eco-morphological, genomic, and epigenomic components of diversity, shedding light on acclimation and adaptation process in these contexts.

Keywords: European whitefish, conservation translocation, geometric morphometric, DNA methylation, epiRADseq
Introduction

Conservation-driven translocations are the intentional, human-mediated movement and release of an organism outside its recorded range, with the aim of establishing new populations to mitigate against the extinction of important conservation units (IUCN & SCC, 2013). Predicted habitat alteration due to climate change, expansion of human activities, and the introduction of invasive species are major factors prompting the use of conservation translocations to preserve biodiversity (Ricketts & Imhoff, 2003; Hoegh-Guldberg et al., 2008; Butchart et al., 2010). Translocations have been shown to improve species conservation status (Hoffman et al., 2010), and are projected to substantially increase as a conservation measure in future years (Swan, Lloyd, & Moehrenschlager, 2018).

Population-level consequences of translocations are expected but the ecological and evolutionary responses poorly understood. Conservation translocations usually consist of small founding population sizes, which can result in failure to capture the genetic diversity of the source population and lead to a loss of genetic diversity and inbreeding (Frankham, Briscoe, & Ballou, 2002; Jamieson, 2011; Furlan et al., 2020). Founder effects can also lead to rapid phenotypic shifts, especially when refuge populations are introduced in areas geographically isolated from the source with no possibility of gene flow (Sendell-Price, Ruegg, & Clegg, 2020). Additionally, refuge populations experience differential selection due to novel environmental pressures, and in some cases have shown rapid genomic adaptation within the first few generations of a translocation (Marques et al., 2018; Laurentino et al., 2020). Unlike natural range expansions or new colonisations by dispersing individuals, the human influence on conservation translocations and the already at-risk status of the populations are expected to have genomic consequences on the evolutionary trajectories that are difficult to predict.

Nevertheless, on short time scales there may be a lag in the evolutionary genomic responses of introduced populations due to factors such as small population sizes, time required for mutations to occur, and time to linkage disequilibrium break down (Reznick et al., 2019). Epigenetics, on the other hand, provides an alternative and faster route to adaptation (Stajic, Perfeito, & Jansen, 2019). Epigenetic states, such as variable DNA methylation levels, change more rapidly than genetic sequence (van der Graaf et al., 2015), represent a measurable molecular marker, and can change in many individuals of a population simultaneously (Angers, Perez, Menicucci, & Leung, 2020). Regardless of whether this is a transient effect, transgenerational, or short-term heritability, it is suggested that epigenomic responses might facilitate population persistence and adaptation to changing environments through phenotypic plasticity and acclimation (Hu & Barrett, 2017; Angers et al., 2020; Dimond & Roberts, 2020).

A growing body of evidence from fishes in particular has shown how exposure to different environmental pressures can affect DNA methylation (Smith, Martin, Nguyen, & Mendelson, 2015; Le Luyer et al., 2017; Gavery, Nichols, Goetz, Middleton, & Swanson, 2018) and this contributes to the
expression of phenotypic variation across different environments (Campos, Valente, Conceição, Engrola, & Fernandes, 2013; Smith, Smith, Kenny, Chaudhuri, & Ritchie, 2015; Artemov et al., 2017). Furthermore, studies have found variation in DNA methylation to exceed that of standing genetic variation in some cases, suggesting a potential compensating role of epigenetics (Richards, Schrey, & Pigliucci, 2012; Schrey et al., 2012) as an alternative route to generating phenotypic plasticity and variation (Angers et al., 2020). The epigenomic responses of natural populations to conservation translocations have rarely been explored but may provide important insight to key early stages of refuge population establishment.

Here we aimed to determine consistent response to translocations at the morphological and molecular level in refuge populations of European whitefish, *Coregonus lavaretus*. In Scotland, the European whitefish (also known as powan) has a native range restricted to only two lakes, Loch Lomond and Loch Eck. These populations were colonised postglacially and are genetically closely related relative to other British populations (Crotti et al., 2020; Crotti et al., in review). Due to concerns for the future of these Scottish populations (Maitland & Lyle, 2013), a series of translocations were carried out over thirty years (Adams et al., 2014) (Figure 1). Between 1988 and 1990 individuals from Loch Lomond were used to establish refuge populations in Loch Sloy and Carron Valley Reservoir. Between 2009 and 2010, fish from Loch Lomond, augmented with a few individuals from Loch Sloy, were used to establish refuge populations in Lochan Shira and Allt na Lairige. Between 2010 and 2011, individuals from Loch Eck were used to establish refuge populations in Loch Tarsan and Loch Glasahan. The first refuge populations (30 years before this study) were established with a much smaller number of families and released individuals compared to the later translocations (7-9 years before this study) (Maitland & Lyle, 2013; Adams et al., 2014) (see Table S1 for detailed information on the number of families, eggs, fry, and adult fish released in each refuge lake). Morphological and some neutral population genetic divergence at microsatellite loci was found between Loch Lomond and the first two translocations (Etheridge et al. 2010; Præbel et al., 2019), suggesting an effect of translocation on evolutionary trajectories that could be concerning for conservation management. The full set of translocations have never been characterised for eco-morphological, genomic, or epigenomic associations with these population establishments in new environments.

Here we used repeated and independent translocations of whitefish populations across a time series to explore the ecological and evolutionary consequences. Repeated translocations from the same source populations provide a rare opportunity to evaluate replication in these processes and also have the potential to inform the management of future translocations (Furlan et al., 2020). Using a combined approach based on ecological, morphological, genomic, and epigenomic analyses, within and across the two source and multiple refuge populations, we: a) quantified phenotypic and ecological trait divergence and convergence; b) assessed genome-wide diversity and differentiation; c) investigated differential genomic responses to selection; and d) investigated parallel response in genome-wide differential DNA methylation levels. Our primary focus was between source and refuge populations with the aim of inferring shared population-level
responses to the conservation measure. Collectively, these analyses provided a comprehensive insight into the molecular, ecological, and evolutionary effects of human-mediated translocations.

Materials and Methods

Sample collections

European whitefish individuals were collected from eight Scottish lochs (Figure 1) in two lake translocation systems: Eck (n = 12 individuals; source), Glashan (n = 34; refuge), Tarsan (n = 33; refuge), which form the Eck translocation system, and Lomond (n = 8; source), Allt na Lairige (n = 9; refuge), Shira (n = 17; refuge), Carron Valley Reservoir (n = 18; refuge), Sloy (n = 17; refuge), which form the Lomond translocation system. Sampling occurred between August and October 2017 using multi-panel, Nordic-pattern gillnets. Fish collection was undertaken under license from Scottish Natural Heritage (now NatureScot) and Marine Scotland. Individuals were photographed on the left side. White muscle tissue from the left side, underneath the dorsal fin and above the lateral line, was taken for genomic and epigenomic analyses and stored in absolute ethanol at -20°C. For stable isotope analysis (SIA), we collected ~1 cm³ of muscle tissue from the right side of the fish, underneath the dorsal fin and above the lateral line, and the stomach contents, and both were stored at -20°C. Due to different sampling schemes, we could not collect stable isotope data from the source population of Eck.

In addition to the samples collected in 2017, we included previously collected samples in the genomic and morphometrics analyses when available. For the genomic analyses we included a subset of the parent fish from Loch Lomond (n = 40), Loch Sloy (n = 17), and Loch Eck (n = 41) that were used to establish the refuge populations between 2009 and 2011. For the morphometric analyses we added photographs from: a sampling of the parent fish from Loch Lomond (n = 89) and Loch Eck (n = 118) that were used to establish the refuge populations between 2009 and 2011; samples from refuge populations Allt na Lairige (n = 4), Lochan Shira (n = 15), Loch Glashan (n = 33), and Loch Tarsan (n = 60) collected during a survey in 2014 and 2015 (Lyle, Stephen, & Adams, 2017); and samples from Loch Lomond (n = 21), Carron Valley Reservoir (n = 11), and Loch Sloy (n = 20) collected in a survey in January 2018.

Geometric morphometric analysis

All photos used for morphometric analyses were taken with the same protocol, on the left sides, using graph paper or ruler for scale. Body shape was captured with 14 fixed landmarks (Figure S1a) chosen based on previous studies and for their established functional importance in foraging and locomotion (Siwertsson, Knudsen, Adams, Præbel, & Amundsen, 2013; Jacobs et al., 2019) (N = 508 individuals). Landmarks were digitised using TPSDig2 v.2.16 (Rohlf, 2010). Statistical analyses were conducted in the R environment (R
Core Team, 2019) with the package geomorph v.3.0.7 (Adams & Otarola-Castillo, 2013). A Generalised Procrustes Analysis was performed to remove variation due to size and orientation of individuals. We tested for homogeneity of allometric curves using the function procD.allometry. The linear model used was $Shape \sim \log(Size) \times Lake$, with $Shape$ being the combination of all principal components, and $Size$ the centroid size (the square root of summed squared distances of landmarks from the configuration centroid).

We implemented the procD.allometry function for each lake system separately. When the interaction term was significant, we performed a pairwise test for homogeneity of slopes using the advanced.procD.lm function, to test if populations differed in allometric slope. If the interaction term was not significant, i.e. if different populations have common or parallel trajectories, we performed pairwise tests for shape difference. Significance was assessed with a randomised residual permutation procedure with 1,000 iterations. We performed a principal component analysis (PCA) on the Procrustes coordinates of all individuals to explore the major axes of variation.

We performed a phenotypic trajectory analysis (PTA) (Collyer & Adams, 2013) in geomorph to quantify the level of parallelism, or deviation from it, in body shape change in response to the translocations across the two lake systems. Significant difference in trajectory direction ($\theta_P$: differences in the direction of phenotypic change) and trajectory lengths ($\Delta L_P$: differences in the magnitude of phenotypic change) was assessed using 1,000 permutations.

**Linear trait analysis**

Linear measurements of nine body traits plus fork length were obtained from distance between landmarks (Figure S1b) (N = 508 individuals). Traits were chosen based on previous publications (Siwertsson et al., 2013; Jacobs et al., 2019) to represent functionally relevant features that respond to differences in diet and environment. Because these linear traits are correlated to fish body length, they were first corrected for allometry following Siwertsson et al. (2013). Briefly, to reduce variance each trait was log10-transformed, then we calculated a common slope for each trait using an ANCOVA with the formula $Trait \sim Lake \times Size$.

The slope was then used to in the formula (Siwertsson et al., 2013):

$$\log_{10} Y_{st} = \log_{10} Y_{obs} + b(\log_{10} L_{st} - \log_{10} L_{obs})$$

Where $Y_{st}$ is the standardised trait value, $Y_{obs}$ is the observed trait value, $b$ is the slope of the ANCOVA, $L_{st}$ is the average length of all whitefish examined, and $L_{obs}$ is the measured body length of each fish.

Divergence in linear traits between lake systems and lakes was then compared using a Kruskall-Wallis test with a post hoc Dunn test with the Benjamini-Hochberg (BH) correction for multiple testing. A PCA was carried out to determine the major axes of phenotypic variation between source and refuge populations, across and within lakes.
Stable Isotope Analysis

Lipid extraction of tissue and stable isotope measurement methods followed Yohannes et al. (2013). Isotopic turnover rate of muscle tissue reflects diet during the preceding 2-4 months (Vander Zanden, Clayton, Moody, Solomon, & Weidel, 2015), while stable isotope of stomach content reflects very recent diet, and by using these two values we could compare how stable diet is in these populations. Muscle tissue was dried in an oven at 50 °C for 48 hours. Briefly, the dried muscle (Glashan = 10, Tarsan = 10, Lomond = 8, Allt na Lairige = 9, Shira = 10, Carron Valley = 9, Sloy = 10) and stomach content (Glashan = 10, Tarsan = 9, Lomond = 6, Allt na Lairige = 8, Shira = 9, Carron Valley = 10, Sloy = 3) samples were immersed in a 2:1 chloroform:methanol solvent with a volume four times that of the sample. Samples were mixed for 30 seconds, rested for 20 minutes, centrifuged for 5 minutes at 3400 rpm, and the supernatant removed. We repeated this process three to four times, until the supernatant was clear. Samples were then rinsed in distilled water and dried at 60°C for 48 hours. Sub-samples of 0.7 – 1 mg were combusted in a vario Micro cube element analyser (Elementar, Analysensysteme, Germany). Stable isotope ratios of carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) were measured with an Isoprime (Micromass, Manchester, UK) isotope ratio mass spectrometer.

SIA was conducted using the framework proposed by Cucherousset & Villegér (2015), using the $si\_div.R$ set of functions (Cucherousset & Villegér, 2015) in R (R Core Team, 2019). For each population we first calculated isotopic richness IRic and isotopic divergence IDiv. Isotopic richness represents the total extent of multidimensional foraging niche space used by populations, i.e. the convex hull area, while isotopic divergence quantifies the distribution of populations within isotopic space, with values of 0 indicating populations are close to the centre of gravity and of 1 when close to the edge of the convex hull. The analyses were run on scaled, unitless (zero to one) coordinates (Cucherousset & Villegér, 2015).

EpiRADseq and ddRADseq library preparation

Samples collected in 2017 were prepared using epiRADseq (Schield et al. 2016) for genomic and epigenomic analyses (Table 1). Genomic SNPs from ddRADseq and epiRADseq are equivalent for estimating genetic diversity and population structure (Crotti, Adams, & Elmer, 2020). The parent fish were prepared using ddRADseq (Peterson et al. 2012) for genomic analyses only, because only their fin tissue was available and DNA methylation is tissue-specific. The protocol used for the ddRADseq and epiRADseq libraries follows Jacobs et al. (2019), with minor modifications described in Crotti et al. (2020). The ddRADseq libraries used $PstI$-HF and $MspI$ enzymes (New England BioLabs) and the epiRADseq libraries used $PstI$-HF and the methylation-sensitive $Hpall$ enzymes. The enzymes $MspI$ and $Hpall$ have the same recognition site. Libraries were sequenced on an Illumina NextSeq500 with 75-bp paired-end reads at Glasgow Polyomics to a depth of 400 M reads each.

This article is protected by copyright. All rights reserved
The epiRADseq dataset was composed of 113 individuals (1.3 M – 15.1 M reads per sample) split among three libraries and the ddRADseq library was composed of 96 individuals (2.5 M – 9.1 M reads per sample) (Table S2).

Genotyping by Sequencing data processing

First, raw reads were demultiplexed with process_radtags in Stacks v.2.4.1 (Rochette, Rivera-Colón, & Catchen, 2019; Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) and trimmed to 65 bp, and both forward and reverse reads were retained. We then trimmed the first 5 and 3 bp with Trimmomatic (Bolger, Lohse, & Usadel, 2014) from the forward and reverse reads to remove the enzyme cut site, and paired-end trimming was done with the following settings: LEADING = 20, TRAILING = 20, to remove low quality reads, and CROP = 60, so that reads were all of the same length. As a reference genome, we used a chromosome-level assembly (GCA_902810595.1) of Coregonus sp. “Balchen” (De-Kayne, Zoller, & Feulner, 2020) which is part of the Alpine lineage of the same European whitefish species complex as the Scottish samples, and split from the Scottish lineage before the last glacial maximum ca 21 K years ago (Hudson et al., 2011; Crotti et al., in revision). Reads were mapped to the genome using bwa mem v.0.7.17 (Li & Durbin, 2009) with default settings and retained if mapping quality was > 20 with samtools v.1.7 (Li et al., 2009). After mapping to the reference genome, samples retained on average 4.1 M reads (SD = 1.9 M). We assembled loci using Stacks v.2.4.1 and the ref_map.pl script. Genotyping in Stacks resulted in a total of 1,234,536 loci, with an average effective per-sample coverage of 11.6x (SD = 4.3x, min = 4.3x, max = 29.9x). A principal component analysis (PCA) revealed the presence of a batch effect between the epiRADseq and ddRADseq libraries on PC2. To identify and exclude the loci responsible, we used two approaches: a) we ran a PCA and calculated the correlation between eigenvectors and SNP genotype using the snpgdsPCACorr function in the R package SNPRelate v. 1.16 (Zheng et al., 2012), and b) we ran a PCA and calculated the loading factor for each SNP on PC2 in the R package adegenet v. 2.1.1 (Jombart, 2008). Loci for which SNPs showed a correlation or loading factor higher than 0.3 were considered as strongly correlated with library type (Ratner, 2009) and added to a blacklist (total number of blacklisted loci = 737) in Stacks and excluded from further analyses.

Genotyping and filtering for genomic analyses

We generated three datasets for population genomic analyses: a combined dataset with all eight populations specifically for outlier analyses, and a dataset for the Eck system (i.e. Eck, Glashan and Tarsan) and for the Lomond system (i.e. Lomond, Allt na Lairige, Shira, Carron Valley Reservoir, Sloy) that were used for the genetic diversity, inbreeding, and relatedness analyses. An initial vcf file was generated for each in populations (part of the Stacks pipeline), with the following criteria: -p 6 (minimum number of populations
genotyped), -r 0.75 (minimum proportion of individuals genotyped per population), --min-maf 0.05 (global minor allele frequency filter), --max-obs-het 0.6 (maximum observed heterozygosity required to process a site at a locus) for the combined dataset, -p 4, -r 0.75, --min-maf 0.05, --max-obs-het 0.6 for the Lomond system dataset, and -p 2, -r 0.75, --min-maf 0.05, --max-obs-het 0.6 for the Eck system dataset. One SNP per locus was retained.

Each dataset was then filtered in vcftools v.0.1.15 (Danecek et al., 2011), retaining SNPs that fulfilled the following criteria: a minimum sequencing depth of 5 per individual, a minimum mean sequencing depth of 8 across individuals, a maximum mean sequencing depth across individuals of 40 (to remove possible repetitive reads), a minor allele frequency (MAF) of 0.05, a 33% missing data threshold. After this step, we excluded individuals with more than 30% missing genotypes. We then removed SNPs out of Hardy-Weinberg equilibrium (HWE) within populations using the script filter_hwe_by_pop.pl (available at https://github.com/jpuritz/dDocent/blob/master/scripts/filter_hwe_by_pop.pl) and with the script pop_missing_filter.sh (available at https://github.com/jpuritz/dDocent/blob/master/scripts/pop_missing_filter.sh) removed sites with more than 33% missing data per population. After filtering, the genomic combined dataset comprised 184 individuals and 5,116 SNPs, the Lomond system dataset comprised 110 individuals and 6,333 SNPs, and the Eck system dataset comprised 77 individuals and 3,712 SNPs.

Prior to the redundancy analysis, the combined dataset was split between populations from the Lomond and Eck system for missing data imputation. We imputed missing data using the LD-kNNi method implemented in Tassel v.5 (Bradbury et al. 2007), based on the 10 closest genotypes using the default settings, and re-merged into a combined dataset using bcftools v.1.8.

Genotyping quality assessment

To assess the quality of the ddRADseq and epiRADseq data for combined genomic analyses we: a) calculated the heterozygous miscall rate, which measures putative genotyping errors by estimating deviation from HWE, with the R package radiator (Gosselin, 2020) and b) calculated rarefied allelic richness with the R package hierfstat v.0.04-22, down-sampling to eight samples per population (Goudet, 2005), using epiRADseq and ddRADseq samples separately for each lake sample that had both epiRADseq and ddRADseq samples (Lomond, Sloy, and Eck). The aim of these tests was to identify any deviations between datasets that would be indicative of genotyping errors, which it would influence downstream analyses. In addition, we estimated the genotyping error rate due to low sequencing coverage in the combined population genomics dataset with the ErrorCount.sh script (https://github.com/jpuritz/dDocent/blob/master/scripts/ErrorCount.sh).
Genetic diversity, relatedness, inbreeding, and differentiation

Summary statistics of genetic diversity (expected heterozygosity $H_E$, observed heterozygosity $H_O$), nucleotide diversity $\pi$, and number of private alleles per population were calculated by the population module of Stacks for each lake system separately. For these analyses, we retained all SNPs present in the loci from the Lomond (9,117 SNPs in total) and Eck (5,249 SNPs in total) system datasets, as these metrics do not need to account for linkage disequilibrium. Genomic measures of pairwise relatedness, $R_{xy}$, and individual inbreeding coefficient, $F_H$, were estimated in Plink v.1.9 (Chang et al. 2015, Purcell et al. 2007) with the make-rel and het functions, respectively, following Waters et al. (2020). Unbiased estimates of inbreeding rely on allele frequencies being derived from an outbred population of unrelated individuals, and with SNPs in linkage equilibrium (Kardos, Luikart, & Allendorf, 2015). Therefore, we further filtered the genomic Lomond and Eck system datasets in Plink, retaining SNPs with $r^2 < 0.2$ within 1 Mb windows, and with a MAF of 0.05 in the source populations, retaining 3,553 in the Lomond system and 2,083 SNPs in the Eck system genomic datasets, respectively. $R_{xy}$ measures the expected proportion of shared alleles between individual pairs that are identical by descent, while $F_H$ compares the observed number of homozygous genotypes to the expected mean number under random mating (Taylor, 2015). Differences in the distribution of pairwise relatedness and inbreeding coefficient between populations were tested with Kolmogorov-Smirnov tests in R (R Core Team, 2019), as the data were not normally distributed.

To gain an insight into the impact of founder size on genetic diversity, inbreeding, and relatedness in refuge populations, we regressed the number of families used to create the refuge populations (Table S1) against the average decrease in observed heterozygosity (in percentage), and average increase in inbreeding and relatedness in the refuge populations in R.

Between population Weir and Cockerham $F_{ST}$ (Weir & Cockerham, 1984) was calculated in GenoDive (Meirmans & Van Tienderenn, 2004) and significance assessed with 10,000 permutations. We employed a maximum-likelihood approach for population assignment with Admixture v.1.3.0 (Alexander, Novembre, & Lange, 2009). We ran analyses with a 20-fold cross-validation (CV), and tested $K$ values ranging 1-5, and the optimal value was defined as the one with the lowest CV-error. Furthermore, we ran a principal component analysis with SNPRelate for all the three datasets. The pairwise $F_{ST}$ and Admixture analyses were run on each lake system dataset separately.

Detection of outliers

To detect genomic outlier SNPs associated with translocations, we used two approaches. First, we applied a redundancy analysis (RDA) to the combined dataset as a multilocus genotype-environment association (GEA) using the R package vegan v.2.5-3 (Oksanen et al., 2018). RDA is a multivariate approach which can simultaneously analyse the response of thousands of genomic variants to predictors of choice and is
thus suitable for genotype-environment association (Forester, Lasky, Wagner, & Urban, 2018). Briefly, RDA uses constrained ordination to model a set of explanatory variables, and unconstrained ordination axes to model the dependent variables (Forester et al., 2018). SNPs that load heavily on one or more explanatory variables are considered outliers. The dependent variable was the multilocus genotype (each genomic SNP) and the explanatory variables were the lake type (source or refuge) and lake system (source population Lomond or Eck). Significance of the RDA was assessed by performing an analysis of variance (ANOVA) with 1,000 permutations. The percentage of variation explained by the RDA ($R^2$) was calculated using the function RsquareAdj in vegan. SNPs with a loading greater than $\pm 2.5$ standard deviation, or z-score, (equivalent to a two-tailed p-value = 0.01) on the lake type RDA axis were considered to be outliers.

Second, we used a Bayesian framework implemented in BayPass (Gautier, 2015). As with the RDA analysis, we looked for an association between genotype and lake type (source or refuge) as a binary covariate using the AUX model. BayPass accounts for confounding demographic effects by estimating a covariance matrix of allele frequencies between populations, so we did not include lake system as a covariate. The AUX model uses Bayes Factors (BFs) to identify SNPs associated with covariates based on a calibration procedure using pseudo-observed datasets (PODs) (Gautier, 2015).

To visualise the location of the outlier SNPs recovered by RDA and BayPass across the genome, we averaged the frequency of the major allele over the two source populations and the six refuge populations respectively, calculated the difference and the relative z-score, and plotted the z-score of the absolute allele frequency change per SNP.

EpiRADseq data processing

EpiRADseq relies on the comparison of read counts to detect loci that are differentially methylated between groups (Schield et al., 2016). To assemble loci, we mapped the quality trimmed fastq files against the genome-referenced Stacks catalogue from the population genomic analysis using bwa mem with default settings. Read counts at each locus were extracted using the samtools idxstats command for all individuals separately, and subsequently combined to create a count table for each lake system separately.

Preliminary analyses using multidimensional scaling (MDS) in the R package edgeR v.3.24.3 (Robinson, McCarthy, & Smyth, 2010) revealed a sequencing library batch effect. It was not possible to incorporate batch effect in the model because individuals from the eight populations were not represented equally across the three epiRADseq libraries. Therefore, we subset the count table to contain only the populations for which individuals were spread across the different libraries and used a negative binomial generalised linear model with the function glmFit in edgeR to identify loci whose read counts were influenced by library. Loci with false discovery rate (FDR) $<$0.05 were then excluded from the count table. After removing the library effect, we identified a weak batch effect associated with the Illumina adapter
barcode. We reiterated the same procedure and excluded loci affected by this bias. Finally, we excluded loci that had non-zero read counts in fewer than 33% of individuals from each lake system separately to remove uninformative loci present in only a small number of individuals. After filtering, the epigenomic Lomond system (N = 66 individuals), Eck system (N = 45), and combined datasets (N = 111) had totals of 120,897, 117,395, and 114,565 loci respectively.

Differential DNA methylation analysis
Differential methylation patterns between source and each refuge population were examined for the Lomond and Eck system separately using the \textit{glmFit} and \textit{glmLRT} functions in \textit{edgeR}. All loci with an FDR < 0.05 for each comparison were considered as differentially methylated (DM). Excess of DM loci sharing between source-refuge population comparisons was calculated with the R package \textit{SuperExactTest v.1.0.7} (Wang, Zhao, & Zhang, 2015).

To explore the major axes of epigenomic variation shared across groups, we log-transformed the read counts with the function \textit{rld} and performed a PCA in \textit{pcaMethods v.1.74} (Stacklies, Redestig, Scholz, Walther, & Selbig, 2007) for the combined dataset and each lake system separately. Additionally, to identify loci with methylation levels associated with lake type (source or refuge), we conducted an RDA on the log-transformed read counts of the combined read count table (dependent variable), using lake type and lake system as explanatory variables (as in the genomic RDA). Loci with z-transformed loading greater than ± 2.5 on the lake type RDA axis were considered to be outliers. Because DNA methylation levels are also heavily influenced by the age of the individual (Angers et al., 2020), we ran a separate RDA with the addition of age as explanatory variable, to assess whether the observed epigenomic patterns were driven by this variable.

Gene ontology analyses
To explore putative functions, we analysed outlier SNPs and DM loci using gene ontology (GO) annotations. Genes overlapping the SNPs and DM loci, and genes within 3000 bp upstream and downstream of these outliers were retained for the analysis. Protein sequences from the European whitefish genome (De-Kayne et al. 2020) were mapped and annotated to the SwissProt database using \textit{Blast2Go} (Götz et al., 2008). Loci were annotated with \textit{BEDTools v.2.27.1} (Quinlan & Hall, 2010) to identify genes and associated proteins from the European whitefish genome. Over-representation tests were conducted in \textit{PANTHER} (Thomas et al., 2003; Mi et al. 2010) using Fisher’s Exact test. Genes were considered as significantly enriched if FDR < 0.1. The set of genes overlapping the STACKS loci was used as background dataset for the GO enrichment analysis.
Genomic and epigenomic association with morphological variation

We aimed to disentangle the association of genomic and epigenomic with morphological variance following translocation, across lake system and age since establishment. To do so, we conducted one RDA and two partial RDAs (pRDAs) to partition the percentage of morphological variation due to genomic and epigenomic effects together, $\text{morpho} \sim \text{gen + epi}$, proportion of morphological variation explained by genomic effects while accounting for epigenomic variation, $\text{morpho} \sim \text{gen + Condition(epi)}$, and proportion of morphological variation explained by epigenomic effects while accounting for genomic variation, $\text{morpho} \sim \text{epi + Condition(gen)}$, following a similar approach by Rougeux, Laporte, Gagnaire, & Bernatchez (2019). We ran these analyses using the first three PCs from the morphological PCA on body shape (comprising 54% of total variation), the PCA on the genomic combined dataset, and the PCA on the epigenomic combined dataset, using all lakes and each lake system separately. We included only the first three PCs from both the genomic and epigenomic PCAs as proportion of variation declines rapidly after PC1 in both analyses (Fig. 3, Fig. S6). Estimation of morphological variance explained by genomic-epigenomic interactive effect was computed with the function \textit{varpart} in \textit{vegan}. Because DNA methylation variation arises more rapidly than genetic variation (van der Graaf et al. 2015), we tested if genomic and epigenomic variation correlated differently with morphological variation at different stages of population divergence. For this, the Lomond system was split into two groups, source and young ($\leq 7$-9 years old) refuge populations, and source and old (30 years old) refuge populations.

Results

Morphological analyses

Morphological analyses revealed a combination of lake specific patterns and some general trends of similarity across refuge populations. Testing homogeneity of allometric slopes showed a significant association between body shape and the interaction between body size and lake of origin for the Eck system ($F_{2,277} = 5.72$, p-value = 0.001), with the refuge populations having different allometric trajectories compared to the source population (Table S3). For the Lomond system, we found a significant interaction between body size and lake when all populations were included in the model ($F_{4,215} = 2.2$, p-value = 0.01), but not when Shira was excluded ($F_{3,191} = 0.85$, p-value = 0.405), indicating that Shira fish had a different allometric trajectory compared to the other populations (Table S3). All other refuge populations from the Lomond system showed similar allometric trajectory but significant body shape differences compared to the source (Table S3).

The PCA of body shape showed there are differences in average body shape within and between systems (Figure 2a, Figure S2a). In the Eck system individuals from the refuge populations had smaller
heads and larger bodies compared to individuals from the source, while in the Lomond system refuge populations had larger heads and smaller bodies compared to the source (Figure 2a). The refuge populations were grouped more closely on PC1 (22% of variation) and PC2 (20% of variation) than the source populations. The next three PCs combined explained 27% of the variation.

The phenotypic change in body shape between source and the combined refuge populations in the two lake systems was similar in magnitude, as inferred from PTA ($\Delta L_p = 0.001$, p-value = 0.4). However, the direction of phenotypic change between source and refuge populations differed significantly across systems ($\theta_p = 96.14^\circ$, p-value = 0.001); the different directions resulted in a convergence of the source to refuge trajectories on PC1 (Figure S2b).

In linear traits, the lakes were generally similar, with only body depth posterior, caudal peduncle length, and fin length were significantly different in most source-refuge population comparisons across lake system (Table S4, Figure S3). The Eck system refuge populations differed from the source population in more body measurements than did the Lomond system (Table S4, Figure S3).

Ecological niche
We found a positive relationship between $\delta^{15}$N from muscle and $\delta^{15}$N from stomach content ($F_{1,5} = 17.03$, p-value < 0.01), with the Loch Lomond and Carron populations showing the highest $\delta^{15}$N levels, Glashan and Tarsan intermediate levels, and Allt na Lairige, Shira, and Sloy the lowest (Figure S2c). This relationship between muscle and stomach content $\delta^{15}$N isotopes indicates that the difference in diet is maintained over time, integrated from food to muscle.

In the Lomond system, isotopic richness (IRic) and isotopic divergence (IDiv) were higher in most refuge populations compared to source (Table S5). This suggests that the diversity of diet of the source population was lower than that of the refuge populations. In the Eck system, there was little difference between the two refuge populations, with Tarsan having slightly higher IRic and IDiv (Table S5). There was no overlap in isotopic niche space between the Lomond population and its refuge populations (Figure 2b).

Genotyping quality assessment
The heterozygous miscall rate was less than 0.1% for the ddRADseq and epiRADseq samples (Table S6). Rarefied allelic richness in each lake was nearly identical regardless of genotyping method (0-1.0% difference; Table S7). Assuming all low depth homozygote genotypes in the genomic combined dataset were errors, the estimated genotyping error rate due to low read depth was 0.03%. Thus the genomic data were concluded to be high quality even when generated from epiRAD or ddRAD libraries (consistent with Crotti et al. 2020).
Genetic diversity

Genetic diversity and heterozygosity were generally lower in the refuge populations than the source; the difference is very small but significant in most cases (Table 1). No refuge population had any private allelic richness while source lakes had some - though few - private alleles (14 private alleles in Eck, two private alleles in Lomond).

Population inbreeding coefficients $F_H$ were higher in the refuge populations; significantly so in all but one instance (Table 2, Figure 3b). Relatedness $R_{xy}$ was higher in the refuge populations than in the source (Table 2, Figure 3b). $R_{xy}$ in the 30 years old refuge populations of the Lomond system were higher than in the 7-9 years old populations (Figure 3b).

There was a trend that diversity might be associated with founding population size. We found that populations with greater numbers of founders had more genetic diversity ($F_{1,4} = 89.47$, p-value < 0.001), and showed lower inbreeding ($F_{1,4} = 76.9$, p-value < 0.001) and lower relatedness ($F_{1,4} = 12.24$, p-value = 0.02) (Figure S3). This co-varies with inbreeding being slightly higher in the two 30 years old refuge populations (Sloy, Carron) relative to the 7-9 years old populations (Figure 3b). Due to the translocation design being a real-world conservation measure rather than an evolutionary experiment, we cannot tease these influences apart more robustly.

Genetic differentiation

The major source of population genomic variation among individuals was clearly by lake system (Eck or Lomond) (PC1 19%) (Figure 3a). Individuals from different populations within the Eck system were not genetically differentiated (i.e. a lack of separation on PC1, PC2, or [not shown] PC3), while in the Lomond system the 30 years old translocated populations (Sloy, Carron) separated from the 7-9 years old populations along PC2. This concurred with admixture analyses, which suggested two genetic clusters ($K = 2$) as the best fitting scenario; the Sloy population significantly differentiated from Lomond, and three clusters as the second-best fitting scenario, with the Carron population further splitting from Lomond but no further genetic structuring by refuge lake (Figure S5). Admixture analysis on the Eck system dataset found no structuring between the source and refuge populations ($K = 1$ as the best-fitting scenario) (Figure S5).

Population-level genetic differentiation was low to moderate between source and refuge populations in both systems. The Eck source population was slightly, albeit significantly, differentiated from refuge populations ($F_{ST} = 0.002-0.003$, p-value < 0.05) and the two refuge populations were not differentiated from each other ($F_{ST} = 0.0001$, p-value > 0.05) (Table 3). In the Lomond system, $F_{ST}$ between source and refuge populations ranged up to 0.030, with all source-refuge comparisons being
significantly different (p-value < 0.05). There was a trend of age effect, with differentiation being higher between the source and two 30 years old refuge populations than the two 7-9 years old ones, and there was no significant differentiation between the two 7-9 refuge populations (Table 3).

Genomic outliers of translocation

We investigated genomic signals of selection due to translocation, defined as regions of the genome consistently identified as outliers between source and refuge populations across lake systems. Using both lake type (source or refuge) and lake system as explanatory variables, the genomic RDA explained 18.7% (adjusted $R^2 = 0.187$) of the total variance ($F_{2,184} = 22.4$, p-value = 0.001). Of this, lake system explained 96.6% on axis 1, and lake type explained 3.4% (Figure 4a), separating source and refuge populations on axis 2. From the RDA, we identified 70 outlier SNPs associated with lake type (Figure 4b). The analysis implemented in BayPass identified 21 outlier SNPs associated with lake type, 14 of which overlapped with the outliers of the RDA analysis (Figure 4b). Forty of the 77 outlier SNPs could be mapped to genes (±3000 bp) in the whitefish reference genome (Table S8). Outlier SNPs from the two approaches were found to be distributed across the genome (Figure 4c).

From the 14 outlier SNPs shared across both approaches (RDA and BayPass), five were found in or near genes and so could be putatively associated to functions (Figure 4b, Table S8). These genes were DnaJ homolog subfamily C member 18 (DnaJC18), ladderlectin (LADD), G protein-regulated inducer of neurite outgrowth 3 (GPRIN3), Atp8b1, and Toll-like receptor 3 (TLR3).

Differential methylation

Across all lakes, 1,294 loci were differentially methylated (DM loci) between source and refuge populations. Most of the variation in the dataset was explained by lake system. Specifically, PC1 (5% of the total variation) separated Eck system from Lomond, and refuge populations separated from the source populations on PC3 (1.8% of the total variation) (Figure S6a,b). The DM loci were distributed mainly in intergenic regions (61-62%) and within genes (29-30%) (Figure S7).

DM loci were unique to each lake system, with only one locus shared across systems (Locus 77123 on chromosome 3). In the Eck system, there were 139 DM loci between Eck and refuge population Tarsan, and 858 DM loci between Eck and refuge population Glashan. Of these, 81 loci overlapped, which was more than expected by chance (p-value < 0.0001, Figure 5b) and 24 were found in or near genes (Table S9). In the Lomond system there was less variation in the number of DM loci between source and refuge populations, ranging between 50 and 204 (Figure 5a). Ten DM loci were shared across all four comparisons, which was more than expected by chance (p-value < 0.0001) (Figure 5a), one of which mapped within a gene (Table S9), DPYSL5.
There were no significantly enriched GO terms (FDR > 0.1) from the genes associated with DM loci (Eck system), but the top 11 GO terms (based on uncorrected p-value < 0.001, fold enrichment = 8.96 – 90.65) included neural functions (e.g. GO:0099536, synaptic signalling) and ion transport (e.g. GO:1901380, negative regulation of potassium ion transport) (Table S10). The gene DPYSL5, which was shared across all Lomond system populations, may have a function in neuronal differentiation and/or axon growth (Ring et al., 2015).

The epigenomic RDA, using lake type and lake system as explanatory variables, explained 3% (adjusted $R^2 = 0.03$) of the total variance ($F_{2,108} = 2.5$, p-value = 0.001), of which lake system separated on the first axis (65%) and lake type on the second (35%) (Figure 5c). We identified 1,493 loci clearly separating source and refuge populations (Figure 5d), of which 486 were found in or near genes. The GO analysis of these 486 loci recovered eighteen GO terms as significantly enriched (FDR < 0.1), and included nervous system development (e.g. GO:0007399, nervous system development; GO:0048699, generations of neurons; GO:0007409, axonogenesis; GO:0061564, axon development), cellular process (GO:0045595, regulation of cell differentiation; GO:0007154, cell communication), and developmental process (GO:0048856, anatomical structure development; GO:0050793, regulation of developmental process) (Table S11). The RDA that included fish age, in addition to lake type and lake system, continued to explain 3% of the total variance. Lake system and lake type were still resolved on axis 1 and 2, explaining 52% and 28% of the variance respectively, while age on axis 3 explained 20% of the variance (Fig. S8). These results indicated that lake type was a more important source of epigenomic variation than fish age, which we used here as a proxy for methylation that is associated with organismal growth, development, and experience.

Genomic and epigenomic associations with eco-morphology

We applied RDA to partition the variance in morphology that was explained by genomic and epigenomic components. When considering the full dataset, genomic and epigenomic effects together explained 18% (adjusted $R^2 = 0.18$) of the variance in body shape ($F_{6,90} = 4.5$, p-value = 0.001). Genomic and epigenomic components separately explained 7% (adjusted $R^2 = 0.07$, $F_{3,93} = 3.6$, p-value = 0.002) and 16% (adjusted $R^2 = 0.16$, $F_{3,93} = 7.02$, p-value = 0.001) of the variance in morphology respectively (Fig. 6). However, when controlling for epigenomic effects, genomic effects did not explain any variation in morphology (adjusted $R^2 = 0.02$, $F_{3,90} = 1.9$, p-value > 0.05), while when controlling for genomic variation, epigenomic variation explained 11% (adjusted $R^2 = 0.11$, $F_{3,90} = 5.01$, p-value = 0.001) of the morphological variance. This pattern differed between systems and refuge population ages (Fig. 6), and suggests that genomic effects may become more relevant for a population with time. In the Eck system analysis, genomic and epigenomic effects together explained 29% (adjusted $R^2 = 0.29$, $F_{6,33} = 3.7$, p-value = 0.001) of the variance.
in body shape, genomic variation explained none (either alone or when controlling for epigenomic variation; \( p \)-values > 0.1), while epigenomic variation explained 27% (adjusted \( R^2 = 0.29, F_{3,36} = 5.9, \ p\text{-value} = 0.001 \)) and 28% (adjusted \( R^2 = 0.28, F_{3,33} = 5.8, \ p\text{-value} = 0.001 \)) of the variation separately and when controlling for genomic variation respectively (Fig. 6). For the Lomond system, morphological variance in Lomond and the younger, i.e. 7-9 years old refuge populations was not explained by either genomic or epigenomic effects (all \( p \)-values > 0.1). In the group containing Lomond and the older, i.e. 30 year old refuge populations, genomic and epigenomic together explained 16% (adjusted \( R^2 = 0.16, F_{6,30} = 2.1, \ p\text{-value} = 0.015 \)) of the variance in morphology, genomic alone and when accounting for epigenomic effects explained 13% (adjusted \( R^2 = 0.13, F_{3,33} = 2.8, \ p\text{-value} = 0.013 \)) and 10% (adjusted \( R^2 = 0.10, F_{3,30} = 2.2, \ p\text{-value} = 0.038 \)) respectively, and epigenomic variation explained none (\( p \)-values > 0.1).

Discussion
By using a robust natural experiment involving multiple human-mediated, purposeful conservation translocations, we found significant changes in populations of European whitefish for eco-morphology, epigenomic, and genomic patterns within few years following translocation. This represents only 2-10 generations (as age at fertility [Brown & Scott, 1994]). We found evidence of convergent morphology and similar extents of change among refuge populations regardless of time since translocation. Coupled with genomic evidence of differential selection pressures on the refuge populations at key genomic regions, we suggest this reflects consistent and rapid response to the shared environmental conditions in the translocation habitats. We identified common DNA methylation responses in refuge populations within and between translocation systems. Finally, we found a stronger correlation between morphological and epigenomic variation in a younger, i.e. 7-9 years old translocated populations (Eck system), but a stronger correlation between morphological and genomic variation in the older, i.e. 30 years old translocated populations (Lomond system). This suggests that the evolutionary responses to a novel environment for conservation translocations in early stages of the establishment (a few generations) may be mediated through plasticity and epigenomic effects but that in (slightly) more established translocations (ca. 10 generations), genomic changes become established.

Ecological consequences of translocation
We observed significant changes in morphology between all refuge populations compared to the source. As shown by the phenotypic trajectory analysis, these changes occurred in a convergent fashion and with similar magnitude, from notably different phenotypes in the source populations of Eck and Lomond to quite similar body shape in the refuge populations across systems. The observed differences in morphology
likely have important consequences for the ecology of these populations (Siwertsson et al. 2013). While whitefish in Lomond and Eck have been shown to feed predominantly on pelagic (zooplankton) and benthic (macroinvertebrate) prey respectively, and display morphological differences typical of variation associated with their respective diets (Etheridge et al., 2012), the refuge populations show intermediate phenotypes. This could be the result of a switch in the prey type utilised, due to the adoption of a more generalist diet, or due to differences in the invertebrate communities between source and refuge lakes. The refuge lakes also differ from source lakes in surface area, depth, and fish communities (Lyle et al., 2017), which have been shown to influence fish morphology (Kahilainen & Østbye, 2006; Siwertsson et al., 2013; Recknagel, Hooker, Adams, & Elmer, 2017), and might explain some of the observed changes in body shape.

While populations of freshwater fishes are known to vary considerably in their morphology associated with the local environment and lake bathymetry (Siwertsson et al., 2013; Recknagel et al., 2017; Jacobs et al., 2020), it is striking that eco-morphology in the novel environments results in similar patterns across translocated populations of whitefish regardless of lake of origin or time since colonisation. Morphological divergence between translocated and source populations has been observed frequently among fish populations (Collyer, Stockwell, Adams, & Reiser, 2007; Michaud, Power, & Kinnison, 2008; Black, Seears, Hollenbeck, & Samollow, 2017). This effect is probably due to both phenotypic plasticity and local adaptation in response to biotic and abiotic differences between source and refuge environments. Previous research showed that fry from the source population Loch Lomond and refuge population of Loch Sloy raised in a common garden show similar phenotypic differences as those observed in wild, adult individuals (Koene, Crotti, Adams, & Elmer, 2019), demonstrating that the phenotypic changes observed in the refuge populations do have a genetic component.

Different environments resulting in a change in diet between source and refuge populations is also suggested by the stable isotope analysis. Refuge populations had the highest isotopic richness and with a greater inter-individual range in $\delta^{13}C$, indicating a wide trophic niche width (Bearhop, Adams, Waldron, Fuller, & MacLeod, 2004), a signal typical of more littoral feeding consumers compared to pelagic ones (France, 1995). In contrast the source population had low isotopic richness (IRic) and isotopic diversity (IDiv), indicating a narrower foraging niche width (Cucherousset & Villegér, 2015). Furthermore, fish from the source population had high $\delta^{15}N$ isotopes values compared to refuge populations, suggesting a higher trophic position and feeding more on pelagic food sources like zooplankton (Syväranta & Jones, 2007), as observed before for these populations (Pomeroy 1991). The lower $\delta^{15}N$ in the refuge populations suggests a diet dominated by littoral macroinvertebrates (Syväranta & Jones, 2007). Because the refuge lakes possess a much reduced fish community compared to the source lakes fish community (Lyle et al., 2017), the expansion in niche width and diet switch observed in the refuge populations may be the result of ecological release (Bolnick et al., 2010), when a colonising species can expand its trophic niche by utilising
new resources that may have been taken by competitors in the original environment. Consequently, our findings show that rapid ecological release may be an important component of conservation management by translocation.

Whitefish are renowned for high levels of polymorphism and adaptive eco-morphologies in body shape, gill rakers, and physiology (Kahlainen & Østbye, 2006; Evans, Chapman, Mitrofanov, & Bernatchez, 2013; Siwertsson et al., 2013; Laporte; Dalziel, Martin, & Bernatchez, 2016). The genetic similarity and shared evolutionary history of the European whitefish species complex at large geographic areas (Rougeux, Gagnaire, & Bernatchez, 2019; Rougeux, Gagnaire, Præbel, Seehausen, & Bernatchez, 2019), despite high levels of local variation at the smaller scale (Rougeux, Bernatchez, & Gagnaire, 2017; Doenz, Bittner, Volanthen, Wagner, & Seehausen, 2018), could suggest the same mechanisms may underlie our findings on rapid divergence following translocation. In fact, genetic and epigenetic foundations for these eco-morpho-physiological traits and their rapid evolution have been shown in other whitefish systems (Laporte et al., 2015; Jacobs et al., 2019; Laporte et al., 2019).

Population genomic consequences of translocation

The observed levels of reduced genetic diversity in all the refuge populations, and their increased inbreeding and relatedness, suggest the population genomic consequences of these translocations to be predictable. The diversity decline was more evident in the 30 years old refuge populations of whitefish, which were established with a much smaller number of families and fewer released individuals, which we suggest meant less starting genetic variation and a stronger bottleneck. In addition, even among the 7-9 years old refuge populations we found an effect of founding group size. For example, refuge populations from the Eck system had less reduction in heterozygosity and inbreeding compared to those of the Lomond system, and Eck system were established with larger founding group size. Our findings indicate that founder size is an important factor when planning conservation translocations (Allendorf & Lundquist, 2003; Szűcs et al., 2017).

Furthermore, we observed low, but significant, genetic differentiation between source and refuge populations, with $F_{ST}$ in the 30 years old translocated populations being an order of magnitude higher compared to the 7-9 years old ones. This also could be due to different founder size and also longer time of divergence and genetic drift (Groombridge et al., 2012; Szűcs et al., 2017). However because we are exploring previous human-induced changes in a limited number of populations, it is not possible to tease apart those influences. These reductions in genetic diversity and heterozygosity, and increases in inbreeding, in the refuge whitefish populations are not extreme, but an assessment of their stability over time and if there is an effect on fitness would be valuable. Genetic monitoring of the refuge populations is
needed out at regular intervals to detect possible genetic diversity loss over time and consider mitigation measures (Schwartz, Luikart, & Waples, 2007).

Consistent signals of local adaptation in refuge populations

Absence of gene flow and selective pressures due to environmental differences can push the evolution of source and refuge populations on separate trajectories (Vincent, Dionne, Kent, Lien, & Bernatchez, 2013). Thus, the use of multiple, independent translocations in this study is a powerful way to gain insights into the process of rapid adaptation to the local environment, and to identify the functional regions under selection during initial population divergence. Across lake systems we found five genes putatively under differential selection in refuge populations compared to source, and that might be involved in local adaptation to the new environments. Two of these genes, ladderlectin and TLR3, are involved in the immune system. TLR3 is an immune receptor specialised in recognising double-stranded RNA viruses (Sahoo et al., 2015), while ladderlectin is a protein involved in pathogen elimination with the ability to bind Gram-negative bacteria and chitin (Russell, Young, Smith, Hayes, & Lumsden, 2008). In addition, DnaJC18, which is part of the Heat shock protein (HSP) family Hsp40, has been found to be upregulated following bacterial infection in catfish (Song et al., 2014) indicating a role in immune system response. Local adaptation in the immune system in response to novel environmental conditions and habitat-specific parasite communities is abundant in freshwater fish (Eizaguirre, Lenz, Kalbe, & Milinski, 2012; Pavey et al., 2013) and consistent with earlier work showing differences in parasite load and infection rate in source compared to the two 30 years old refuge populations of whitefish (Etheridge et al. 2010). Because we found these genes across all refuge populations, it suggests an aspect of identical molecular responses to selection pressures in the immune response. Other relevant candidate genes included involvement in nervous system development, such as GPRIN3, a gene active in the physiology of the striatum, a part of the brain involved in the motor system (Karadurmus et al., 2019), and Atp8b1, a lipid metabolism transport gene whose mutations are associated with cholestasis liver disease (Pham, Zhang, & Yin, 2017). Overall, genomic outliers shared by refuge populations suggest immune response, nervous system, and metabolism functions are among the first to be impacted and strongly under selection when fishes colonise and adapt to new environments (Elmer et al., 2010; Terekhanova et al., 2014; Vatsiou, Bazin, & Gaggiotti, 2016; Marques et al., 2018). This may reflect new evolutionary trajectories in translocated populations.

Epigenomic consequences of translocation

DNA methylation, and epigenetic mechanisms in general, provide a molecular route to phenotypic plasticity (Angers et al., 2020), which plays a central role in facilitating the establishment and persistence of populations in new habitats (Lande, 2015). We detected shared differentially methylated loci between
source and refuge populations across lake systems, reflecting consistent response by the epigenome to translocation. Several differentially methylated loci were in or near genes involved in neural functions. For example, DPYSL5, is involved in neural development and research showed it had reduced expression in rainbow trout (*Oncorhynchus mykiss*) offspring from thermally stressed mothers, with their fear-related locomotor response and spatial learning abilities impaired (Colson et al., 2019). Candidate gene ZNF367 is a core regulating gene during brain development in teleost fish (Baumgart et al., 2104). Another identified locus was near the gene SYN3, which plays an important role in early neural differentiation and in neuronal progenitor cell development (Garbarino, Costa, Pestarino, and Candiani, 2014), and in a salmonid-wide analysis was found under intensified and diversifying selection in the genus *Coregonus* (Schneider, Adams, & Elmer, 2019). However, caution is needed in interpreting any functional role of the differentially methylated loci because of the reduced representation approach we applied and because we examined terminal tissues with unknown link between its methylation and developmental consequences.

DNA methylation is a complex mechanism with most influence being due to the role of differentially methylated regions rather than analysis of single loci, as most epigenetic variants not being deterministic epi-alleles with defined location and effects, but interactive regulatory factors (van der Graaf et al., 2015; Adrian-Kalchhauser et al., 2020). Rather than aiming to definitively identify functional molecular consequences of methylation, the motivation of our experiment was to infer if there is population-level signal of epigenomic response to translocation and if it holds ecologically and evolutionarily valuable signal, thereby prompt future research to examine in more molecular and developmental detail. Indeed, we showed that conservation translocations lead to significant changes in patterns of DNA methylation. Our results are consistent with a potential role of epigenomic variation in adaptation to novel environments that warrants further study. Future high-density genome-wide research of methylation would be valuable for inferring functional targets and responses across translocation environments.

This effect is evident in our finding that lake type (i.e. being refuge or source) explained substantially more variance in epigenomic models than it did in the genomic models (35% vs 3% of the variance captured in the RDA). This agrees with previous findings that individuals reared in different environments exhibit higher epigenomic differentiation than genomic differentiation due to phenotypic plasticity (Artemov et al., Le Luyer et al., 2017; Gavery et al., 2018), suggesting a rapid and strong effect of environments on DNA methylation. Furthermore, we found some evidence that epigenomic variation had stronger association with morphological variation in younger, less genetically differentiated refuge populations (35%), while genetic variation stronger association with morphological variance in the older, more genetically differentiated refuge populations (16%). However, the morphological variation explained by either genomic or epigenomic variation was low in all comparisons, consistent with a previous study looking at the influence of these two factors on gene expression (Rougeux et al., 2019). This suggests that
phenotypic changes in translocated populations might be influenced by other factors that remain to be evaluated and warrant further exploration.

Conclusions
We identified consistent ecological and morphological responses in whitefish refuge populations, suggesting that the ecological and evolutionary consequences of these conservation-driven translocations might be predictable. In the refuge populations, genetic diversity was reduced while relatedness and inbreeding increased; this was related to both the number of founder individuals and time since translocation and are difficult to separate. Genomic and epigenomic analyses suggested roles of neural development, immune system, and metabolism in response to translocation. This demonstrates that transgenerational molecular mechanisms might facilitate acclimation and rapid adaptation to new environments and response to divergent selection that accompany these human-mediated colonisations. In addition, our findings suggest that translocated populations can adapt to their new environments at the genomic level despite a reduction in diversity. Our findings shed light on processes behind recent and rapid differentiation, acclimation, and adaptation in populations of high conservation concern and targets of management effort. This highlights the value of combining genomic and epigenomic approaches to understand ecological and evolutionary responses to novel environments, but also highlight the need for experimental work to better understand the role, and potential transgenerational effect, of epigenomic mechanisms.

Acknowledgments
This work was funded by University of Glasgow College of Medical, Veterinary and Life Sciences doctoral training programme and Wellcome Trust ISSF 097821/Z/11/Z. We thank P Koene, H Honkanen, M Newton, J Fordyce, A Jacobs, and H Recknagel for their assistance with fieldwork; Glasgow Polyomics and J Galbraith for NGS sequencing; A Adam and M Capstick for support in the laboratory; and A Jacobs and H Recknagel for advice on design and analysis. We thank C Bean and the then Scottish Natural Heritage (now NatureScot) for permits.
Data Availability Statement

The data that support the findings of this study, and the scripts used, are openly available in University of Glasgow Enlighten Repository [doi: 10.5525/gla.researchdata.1078]. Short read data are available at NCBI SRA [PRJNA658243].
References

Adams, C. E., Lyle, A. A., Dodd, J. A., Bean, C. W., Winfield, I. J., Gowans, A. R. D., Stephen, A., & Maitland, P. S. (2014). Translocation as a conservation tool: case studies from rare freshwater fishes in Scotland. *The Glasgow Naturalist*, 26(1), 17–24.

Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An r package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, 4(4), 393–399. https://doi.org/10.1111/2041-210X.12035

Adrian-Kalchhauser, I., Sultan, S. E., Shama, L. N. S., Spence-Jones, H., Tiso, S., Keller Valsecchi, C. I., & Weissing, F. J. (2020). Understanding “Non-genetic” Inheritance: Insights from Molecular-Evolutionary Crosstalk. *Trends in Ecology and Evolution* 35(12), 1078–1089. https://doi.org/10.1016/j.tree.2020.08.011

Ahlroth, P., Alatalo, R. V., Holopainen, A., Kumpulainen, T., & Suhonen, J. (2003). Founder population size and number of source populations enhance colonization success in waterstriders. *Oecologia*, 137(4), 617–620. https://doi.org/10.1007/s00442-003-1344-y

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology*, 17(1), 24–30. https://doi.org/10.1046/j.1523-1739.2003.02365.x

Angers, B., Perez, M., Menicucci, T., & Leung, C. (2020). Sources of epigenetic variation and their applications in natural populations. *Evolutionary Applications*, eva.12946. https://doi.org/10.1111/eva.12946

Artemov, A. V., Mugue, N. S., Rastorguev, S. M., Zhenilo, S., Mazur, A. M., Tsygankova, S. V., Boulygina, E. S., Kaplun, D., Nedoluzhko, A. V., Medvedeva, Y. A., & Prokhortchouk, E. B.
(2017). Genome-Wide DNA Methylation Profiling Reveals Epigenetic Adaptation of Stickleback to Marine and Freshwater Conditions. *Molecular Biology and Evolution, 34*(9), 2203–2213. https://doi.org/10.1093/molbev/msx156

Baumgart, M., Groth, M., Priebe, S., Savino, A., Testa, G., Dix, A., Ripa, R., Spallotta, F., Gaetano, C., Ori, M., Terzibasi Tozzini, E., Guthke, R., Platzer, M., & Cellerino, A. (2014). RNA-seq of the aging brain in the short-lived fish *N. furzeri* - conserved pathways and novel genes associated with neurogenesis. *Aging Cell, 13*(6), 965–974. https://doi.org/10.1111/acel.12257

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: A novel approach using stable isotope analysis. *Journal of Animal Ecology, 73*(5), 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Black, A. N., Seears, H. A., Hollenbeck, C. M., & Samollow, P. B. (2017). Rapid genetic and morphologic divergence between captive and wild populations of the endangered Leon Springs pupfish, *Cyprinodon bovinus*. *Molecular Ecology, 26*(8), 2237–2256. https://doi.org/10.1111/mec.14028

Bolnick, D. I., Ingram, T., Stutz, W. E., Snowberg, L. K., Lau, O. L., & Paull, J. S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences, 277*(1689), 1789–1797. https://doi.org/10.1098/rspb.2010.0018

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics, 23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Brown, E. A. R., & Scott, D. B. C. (1994). Life histories of the powan, *Coregonus lavaretus* (L.) (Salmonidae, Coregoninae) of Loch Lomond and Loch Eck. *Hydrobiologia, 290*(1–3), 121–133. https://doi.org/10.1007/BF00008959

Butchard, S. H. M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P. W., Almond, R. E. A., Baillie, J. E. M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K. E., Carr, G. M.,

This article is protected by copyright. All rights reserved
Chanson, J., Chenery, A. M., Csirke, J., Davidson, N. C., Dentener, F., Foster, M., Galli, A., … Watson, R. (2010). Global biodiversity: Indicators of recent declines. *Science, 328*(5982), 1164–1168. https://doi.org/10.1126/science.1187512

Campos, C., Valente, L. M. P., Conceição, L. E. C., Engrola, S., & Fernandes, J. M. O. (2013). Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics, 8*(4). https://doi.org/10.4161/epi.24178

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology, 22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience, 4*(1), 7. https://doi.org/10.1186/s13742-015-0047-8

Collyer, M. L., & Adams, D. C. (2013). Phenotypic trajectory analysis: Comparison of shape change patterns in evolution and ecology. *Hystrix, 24*(1). https://doi.org/10.4404/hystrix-24.1-6298

Collyer, M. L., Stockwell, C. A., Adams, D. C., & Reiser, M. H. (2007). Phenotypic plasticity and contemporary evolution in introduced populations: Evidence from translocated populations of white sands pupfish (*Cyprinodon tularosa*). *Ecological Research, 22*(6), 902–910. https://doi.org/10.1007/s11284-007-0385-9

Colson, V., Cousture, M., Damasceno, D., Valotaire, C., Nguyen, T., Le Cam, A., & Bobe, J. (2019). Maternal temperature exposure impairs emotional and cognitive responses and triggers dysregulation of neurodevelopment genes in fish. *PeerJ, 2019*(1), e6338. https://doi.org/10.7717/peerj.6338

Crotti, M., Adams, C. E., & Elmer, K. R. (2020). Population genomic SNPs from epigenetic RADs: Gaining genetic and epigenetic data from a single established next-generation
sequencing approach. *Methods in Ecology and Evolution, 11*(7), 839–849. 
https://doi.org/10.1111/2041-210X.13395

Cucherousset, J., & Villéger, S. (2015). Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. *Ecological Indicators, 56*, 152–160. 
https://doi.org/10.1016/j.ecolind.2015.03.032

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics, 27*(15), 2156–2158. 
https://doi.org/10.1093/bioinformatics/btr330

De-Kayne, R., Zoller, S., & Feulner, P. G. D. (2020). A de novo chromosome-level genome assembly of *Coregonus sp. “Balchen”*: one representative of the Swiss Alpine whitefish radiation. *Molecular Ecology Resources, 1755-0998.13187*. https://doi.org/10.1111/1755-0998.13187

Dimond, J. L., & Roberts, S. B. (2020). Convergence of DNA Methylation Profiles of the Reef Coral *Porites astreoides* in a Novel Environment. *Frontiers in Marine Science, 6*, 792. 
https://doi.org/10.3389/fmars.2019.00792

Doenz, C. J., Bittner, D., Vonlanthen, P., Wagner, C. E., & Seehausen, O. (2018). Rapid buildup of sympatric species diversity in Alpine whitefish. *Ecology and Evolution, 8*(18), 9398–9412. 
https://doi.org/10.1002/ece3.4375

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. *Ecology Letters, 15*(7), 723–731. https://doi.org/10.1111/j.1461-0248.2012.01791.x

Elmer, K. R., Fan, S., Gunter, H. M., Jones, J. C., Boekhoff, S., Kuraku, S., & Meyer, A. (2010). Rapid evolution and selection inferred from the transcriptomes of sympatric crater lake cichlid fishes. *Molecular Ecology, 19*(1), 197–211. https://doi.org/10.1111/j.1365-294X.2009.04488.x
Etheridge, E. C., Bean, C. W., Maitland, P. S., & Adams, C. E. (2010). Morphological and ecological responses to a conservation translocation of powan (Coregonus lavaretus) in Scotland. Aquatic Conservation: Marine and Freshwater Ecosystems, 20(3), 274–281. https://doi.org/10.1002/aqc.1101

Etheridge, E. C., Bean, C. W., Maitland, P. S., Ballantyne, S., & Adams, C. E. (2012). Discontinuous infraspecific variation in ecological and morphological traits has consequences for conservation of powan (Coregonus lavaretus) in Scotland. Advances in Limnology, 505–517. https://doi.org/10.1127/ADVLIM/63/2012/505

Etheridge, E. C., Bean, C. W., & Adams, C. E. (2011). An experimental approach to estimating vulnerability of European whitefish (Coregonus lavaretus) ova to predation by invasive ruffe (Gymnocephalus cernuus). Ecology of Freshwater Fish, 20(2), 299–307. https://doi.org/10.1111/j.1600-0633.2011.00496.x

Evans, M. L., Chapman, L. J., Mitrofanov, I., & Bernatchez, L. (2013). Variable extent of parallelism in respiratory, circulatory, and neurological traits across lake whitefish species pairs. Ecology and Evolution, 3(3), 546–557. https://doi.org/10.1002/ece3.469

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. Molecular Ecology, 27(9), 2215–2233. https://doi.org/10.1111/mec.14584

France, R. L. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography, 40(7), 1310–1313. https://doi.org/10.4319/lo.1995.40.7.1310

Frankham, R., Briscoe, D. A., & Ballou, J. D. (2002). Introduction to Conservation Genetics. Cambridge University Press.

Furlan, E. M., Gruber, B., Attard, C. R. M., Wager, R. N. E., Kerezsy, A., Faulks, L. K., Beheregaray, L. B., & Unmack, P. J. (2020). Assessing the benefits and risks of translocations in depauperate species: A theoretical framework with an empirical validation. Journal of Applied Ecology, 57(4), 831–841. https://doi.org/10.1111/1365-2664.13581
Garbarino, G., Costa, S., Pestarino, M., & Candiani, S. (2014). Differential expression of synapsin genes during early zebrafish development. *Neuroscience, 280*, 351–367. https://doi.org/10.1016/j.neuroscience.2014.09.015

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics, 201*(4), 1555–1579. https://doi.org/10.1534/genetics.115.181453

Gavery, M. R., Nichols, K. M., Goetz, G. W., Middleton, M. A., & Swanson, P. (2018). Characterization of genetic and epigenetic variation in sperm and red blood cells from adult hatchery and natural-origin steelhead, *Oncorhynchus mykiss*. *G3: Genes, Genomes, Genetics,* 8(11), 3723–3736. https://doi.org/10.1534/g3.118.200458

Gosselin, T. (2020). radiator: RADseq Data Exploration, Manipulation and Visualization using R. *R package version 1.1.9*. https://thierrygosselin.github.io/radiator/authors.html

Götz, S., García-Gómez, J. M., Terol, J., Williams, T. D., Nagaraj, S. H., Nueda, M. J., Robles, M., Talón, M., Dopazo, J., & Conesa, A. (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research, 36*(10), 3420–3435. https://academic.oup.com/nar/article/36/10/3420/2410320

Groombridge, J. J., Raisin, C., Bristol, R., & Richardson, D. S. (2012). Genetic Consequences of Reintroductions and Insights from Population History. In *Reintroduction Biology* (pp. 395–440). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444355833.ch12

Günther, T., & Coop, G. (2013). Robust identification of local adaptation from allele frequencies. *Genetics, 195*(1), 205–220. https://doi.org/10.1534/genetics.113.152462

He, X., Johansson, M. L., & Heath, D. D. (2016). Role of genomics and transcriptomics in selection of reintroduction source populations. *Conservation Biology, 30*(5), 1010–1018. https://doi.org/10.1111/cobi.12674

This article is protected by copyright. All rights reserved
Hoegh-Guldberg, O., Hughes, L., McIntyre, S., Lindenmayer, D. B., Parmesan, C., Possingham, H. P., & Thomas, C. D. (2008). Ecology: Assisted colonization and rapid climate change. *Science, 321*(5887), 345–346. https://doi.org/10.1126/science.1157897

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H. M., Carpenter, K. E., Chanson, J., Collen, B., Cox, N. A., Darwall, W. R. T., Dulvy, N. K., Harrison, L. R., Katariya, V., Pollock, C. M., Quader, S., Richman, N. I., Rodrigues, A. S. L., Tognelli, M. F., … Stuart, S. N. (2010). The impact of conservation on the status of the world’s vertebrates. *Science, 330*(6010), 1503–1509. https://doi.org/10.1126/science.1194442

Hu, J., & Barrett, R. D. H. (2017). Epigenetics in natural animal populations. *Journal of Evolutionary Biology, 30*(9), 1612–1632. https://doi.org/10.1111/jeb.13130

Hu, J., Askary, A. M., Thurman, T. J., Spiller, D. A., Palmer, T. M., Pringle, R. M., & Barrett, R. D. H. (2019). The Epigenetic Signature of Colonizing New Environments in Anolis Lizards. *Molecular Biology and Evolution, 36*(10), 2165–2170. https://doi.org/10.1093/molbev/msz133

Hudson, A. G., Vonlanthen, P., & Seehausen, O. (2011). Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *Proceedings of the Royal Society B: Biological Sciences, 278*(1702), 58–66. https://doi.org/10.1098/rspb.2010.0925

IUCN/SCC. (2013). *Guidelines for Reintroductions and Other Conservation Translocations: Vol. Version 1.*

Jacobs, A., Carruthers, M., Eckmann, R., Yohannes, E., Adams, C. E., Behrmann-Godel, J., & Elmer, K. R. (2019). Rapid niche expansion by selection on functional genomic variation after ecosystem recovery. *Nature Ecology and Evolution, 3*(1), 77–86. https://doi.org/10.1038/s41559-018-0742-9

Jacobs, A., Carruthers, M., Yurchenko, A., Gordeeva, N. V., Alekseyev, S. S., Hooker, O., Leong, J. S., Minkley, D. R., Rondeau, E. B., Koop, B. F., Adams, C. E., & Elmer, K. R. (2020). Parallelism in eco-morphology and gene expression despite variable evolutionary and
genomic backgrounds in a Holarctic fish. *PLoS Genetics, 16*(4), e1008658.
https://doi.org/10.1371/journal.pgen.1008658

Jamieson, I. G. (2011). Founder Effects, Inbreeding, and Loss of Genetic Diversity in Four Avian Reintroduction Programs. *Conservation Biology, 25*(1), 115–123.
https://doi.org/10.1111/j.1523-1739.2010.01574.x

Johnson, K. M., & Kelly, M. W. (2020). Population epigenetic divergence exceeds genetic divergence in the Eastern oyster *Crassostrea virginica* in the Northern Gulf of Mexico. *Evolutionary Applications, 13*(5), 945–959. https://doi.org/10.1111/eva.12912

Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics, 24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Kahilainen, K., & Østbye, K. (2006). Morphological differentiation and resource polymorphism in three sympatric whitefish *Coregonus lavaretus* (L.) forms in a subarctic lake. *Journal of Fish Biology, 63–79*. https://doi.org/10.1111/j.1095-8649.2005.00876.x

Karadurmus, D., Rial, D., De Backer, J. F., Communi, D., de Kerchove d’Exaerde, A., & Schiffmann, S. N. (2019). GPRIN3 Controls Neuronal Excitability, Morphology, and Striatal-Dependent Behaviors in the Indirect Pathway of the Striatum. *The Journal of Neuroscience, 39*(38), 7513–7528. https://doi.org/10.1523/JNEUROSCI.2454-18.2019

Kardos, M., Luikart, G., & Allendorf, F. W. (2015). Measuring individual inbreeding in the age of genomics: Marker-based measures are better than pedigrees. *Heredity, 115*(1), 63–72. https://doi.org/10.1038/hdy.2015.17

Koene, J. P., Crotti, M., Elmer, K. R., & Adams, C. E. (2019). Differential selection pressures result in a rapid divergence of donor and refuge populations of a high conservation value freshwater fish *Coregonus lavaretus* (L.). *Evolutionary Ecology, 33*(4), 533–548. https://doi.org/10.1007/s10682-019-09995-y

Lande, R. (2015). Evolution of phenotypic plasticity in colonizing species. *Molecular Ecology, 24*(9), 2038–2045. https://doi.org/10.1111/mec.13037

This article is protected by copyright. All rights reserved
Laporte, M., Le Luyer, J., Rougeux, C., Dion-Côté, A. M., Krick, M., & Bernatchez, L. (2019). DNA methylation reprogramming, TE derepression, and postzygotic isolation of nascent animal species. *Science Advances, 5*(10), eaaw1644. https://doi.org/10.1126/sciadv.aaw1644

Laporte, M., Dalziel, A. C., Martin, N., & Bernatchez, L. (2016). Adaptation and acclimation of traits associated with swimming capacity in Lake Whitefish (*Coregonus clupeaformis*) ecotypes. *BMC Evolutionary Biology, 16*(1), 1–13. https://doi.org/10.1186/s12862-016-0732-y

Laporte, M., Rogers, S. M., Dion-Côté, A. M., Normandeau, E., Gagnaire, P. A., Dalziel, A. C., Chebib, J., & Bernatchez, L. (2015). RAD-QTL mapping reveals both genome-level parallelism and different genetic architecture underlying the evolution of body shape in lake Whitefish (*Coregonus clupeaformis*) species pairs. *G3: Genes, Genomes, Genetics, 5*(7), 1481–1491. https://doi.org/10.1534/g3.115.019067

Laurentino, T. G., Moser, D., Roesti, M., Ammann, M., Frey, A., Ronco, F., Kueng, B., & Berner, D. (2020). Genomic release-recapture experiment in the wild reveals within-generation polygenic selection in stickleback fish. *Nature Communications, 11*(1), 1–9. https://doi.org/10.1038/s41467-020-15657-3

Law, J. A., & Jacobsen, S. E. (2010, March 9). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Reviews Genetics, 11*(3), 204–220. https://doi.org/10.1038/nrg2719

Le Luyer, J., Laporte, M., Beacham, T. D., Kaukinen, K. H., Withler, R. E., Leong, J. S., Rondeau, E. B., Koop, B. F., & Bernatchez, L. (2017). Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. *Proceedings of the National Academy of Sciences of the United States of America, 114*(49), 12964–12969. https://doi.org/10.1073/pnas.1711229114

Lea, A. J., Altman, J., Alberts, S. C., & Tung, J. (2016). Resource base influences genome-wide DNA methylation levels in wild baboons (*Papio cynocephalus*). *Molecular Ecology, 25*(8), 1681–1696. https://doi.org/10.1111/mec.13436

This article is protected by copyright. All rights reserved
Lenz, T. L., Eizaguirre, C., Rotter, B., Kalbe, M., & Milinski, M. (2013). Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-wide digital gene expression analysis. *Molecular Ecology, 22*(3), 774–786. https://doi.org/10.1111/j.1365-294X.2012.05756.x

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics, 25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Maitland, P. S., & Lyle, A. A. (2013). Ex situ and in situ approaches, including assisted reproduction, for the conservation of native species of charr (Salmonidae) and whitefish (Coregonidae) in Scotland. *International Zoo Yearbook, 47*(1), 129–139. https://doi.org/10.1111/j.1748-1090.2012.00192.x

Marques, D. A., Jones, F. C., Di Palma, F., Kingsley, D. M., & Reimchen, T. E. (2018). Experimental evidence for rapid genomic adaptation to a new niche in an adaptive radiation. *Nature Ecology and Evolution, 2*(7), 1128–1138. https://doi.org/10.1038/s41559-018-0581-8

Meirmans, P. G., & Van Tienderen, P. H. (2004). genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes, 4*(4), 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x

Mi, H., Dong, Q., Muruganujan, A., Gaudet, P., Lewis, S., & Thomas, P. D. (2009). PANTHER version 7: Improved phylogenetic trees, orthologs and collaboration with the Gene Ontology Consortium. *Nucleic Acids Research, 38*(SUPPL.1), 204–210. https://doi.org/10.1093/nar/gkp1019

Michaud, W. K., Power, M., & Kinnison, M. T. (2008). Trophically mediated divergence of Arctic charr (*Salvelinus alpinus* L.) populations in contemporary time. *Evolutionary Ecology Research, 10*(7), 1051–1066.
Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O’Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Szoecs, E., & Wagner, H. (2018). vegan: Community Ecology Package. R package version 2.5-3. https://cran.r-project.org/web/packages/vegan/index.html

Pavey, S. A., Sevellec, M., Adam, W., Normandeau, E., Lamaze, F. C., Gagnaire, P.-A., Filteau, M., Hebert, F. O., Maaroufi, H., & Bernatchez, L. (2013). Nonparallelism in MHCIIβ diversity accompanies nonparallelism in pathogen infection of lake whitefish (Coregonus clupeaformis) species pairs as revealed by next-generation sequencing. Molecular Ecology, 22(14), 3833–3849. https://doi.org/10.1111/mec.12358

Perrier, C., Rougemont, Q., & Charmantier, A. (2020). Demographic history and genomics of local adaptation in blue tit populations. Evolutionary Applications, 13(6), 1145–1165. https://doi.org/10.1111/eva.13035

Pham, D. H., Zhang, C., & Yin, C. (2017). Using Zebrafish to Model Liver Diseases-Where Do We Stand? In Current Pathobiology Reports (Vol. 5, Issue 2, pp. 207–221). Springer. https://doi.org/10.1007/s40139-017-0141-y

Pomeroy, P. P. (1991). A comparative assessment of temporal variation in diet of powan, Coregonus lavaretus (L.), from Loch Lomond and Loch Eck, Scotland, U.K. Journal of Fish Biology, 38(3), 457–478. https://doi.org/10.1111/j.1095-8649.1991.tb03133.x

Praebel, K., Bean, C., Dodd, J., Etheridge, E., Gowans, A., Knudsen, R., Lyle, A., Maitland, P., Winfield, I., & Adams, C. (2019). Allelic Losses and Gains During Translocations of a High Conservation Value Fish, Coregonus lavaretus. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.3358962

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., De Bakker, P. I. W., Daly, M. J., & Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics, 81(3), 559–575. https://doi.org/10.1086/519795

This article is protected by copyright. All rights reserved
Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, 26(6), 841–842. https://academic.oup.com/bioinformatics/article/26/6/841/244688

R Core Team. (2019). *The R Project for Statistical Computing*. Http://Www.R-Project.Org/. https://www.r-project.org/

Ratner, B. (2009). The correlation coefficient: Its values range between 1/1, or do they. *Journal of Targeting, Measurement and Analysis for Marketing*, 17(2), 139–142. https://doi.org/10.1057/jt.2009.5

Recknagel, H., Hooker, O. E., Adams, C. E., & Elmer, K. R. (2017). Ecosystem size predicts eco-morphological variability in a postglacial diversification. *Ecology and Evolution*, 7(15), 5560–5570. https://doi.org/10.1002/ece3.3013

Reznick, D. N., Bassar, R. D., Handelsman, C. A., Ghalambor, C. K., Arendt, J., Coulson, T., Potter, T., Ruell, E. W., Torres-Dowdall, J., Bentzen, P., & Travis, J. (2019). Eco-Evolutionary Feedbacks Predict the Time Course of Rapid Life-History Evolution. *The American Naturalist*, 194(5), 671–692. https://doi.org/10.1086/705380

Richards, C. L., Schrey, A. W., & Pigliucci, M. (2012). Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecology Letters*, 15(9), 1016–1025. https://doi.org/10.1111/j.1461-0248.2012.01824.x

Ricketts, T., & Imhoff, M. (2003). Conservation Ecology: Biodiversity, Urban Areas, and Agriculture: Locating Priority Ecoregions for Conservation. *Conservation Ecology*, 8(2), 1. https://doi.org/10.2307/26271982

Ring, K. L., An, M. C., Zhang, N., O’Brien, R. N., Ramos, E. M., Gao, F., Atwood, R., Bailus, B. J., Melov, S., Mooney, S. D., Coppola, G., & Ellerby, L. M. (2015). Genomic Analysis Reveals Disruption of Striatal Neuronal Development and Therapeutic Targets in Human Huntington’s Disease Neural Stem Cells. *Stem Cell Reports*, 5(6), 1023–1038. https://doi.org/10.1016/j.stemcr.2015.11.005

This article is protected by copyright. All rights reserved
Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Rochette, N. C., Rivera-Colón, A. G., & Catchen, J. M. (2019). Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. *Molecular Ecology, 28*(21), 4737–4754. https://doi.org/10.1111/mec.15253

Rohlf, F. J. (2010). *tpsUtil v 2.16 & tpsDig v 2.16*. SUNY.

Rougeux, C., Bernatchez, L., & Gagnaire, P. A. (2017). Modeling the multiple facets of speciation-with-gene-flow toward inferring the divergence history of lake whitefish species pairs (*Coregonus clupeaformis*). *Genome Biology and Evolution, 9*(8), 2057–2074. https://doi.org/10.1093/gbe/evx150

Rougeux, C., Gagnaire, P., & Bernatchez, L. (2019). Model-based demographic inference of introgression history in European whitefish species pairs’. *Journal of Evolutionary Biology, 32*(8), 806–817. https://doi.org/10.1111/jeb.13482

Rougeux, C., Gagnaire, P., Praebel, K., Seehausen, O., & Bernatchez, L. (2019). Polygenic selection drives the evolution of convergent transcriptomic landscapes across continents within a Nearctic sister species complex. *Molecular Ecology, 28*(19), 4388–4403. https://doi.org/10.1111/mec.15226

Rougeux, C., Laporte, M., Gagnaire, P.-A., & Bernatchez, L. (2019). The role of genomic vs. epigenomic variation in shaping patterns of convergent transcriptomic variation across continents in a young species complex. *BioRxiv, 784231*. https://doi.org/10.1101/784231

Russell, S., Young, K. M., Smith, M., Hayes, M. A., & Lumsden, J. S. (2008). Cloning, binding properties, and tissue localization of rainbow trout (*Oncorhynchus mykiss*) ladder lectin. *Fish and Shellfish Immunology, 24*(6), 669–683. https://doi.org/10.1016/j.fsi.2007.11.002

Schild, D. R., Walsh, M. R., Card, D. C., Andrew, A. L., Adams, R. H., & Castoe, T. A. (2016). EpiRADseq: scalable analysis of genomewide patterns of methylation using next-generation
sequencing. *Methods in Ecology and Evolution*, 7(1), 60–69. https://doi.org/10.1111/2041-210X.12435

Schneider, K., Adams, C. E., & Elmer, K. R. (2019). Parallel selection on ecologically relevant gene functions in the transcriptomes of highly diversifying salmonids. *BMC Genomics*, 20(1), 1–23. https://doi.org/10.1186/s12864-019-6361-2

Schrey, A. W., Coon, C. A. C., Grispo, M. T., Awad, M., Imboma, T., McCoy, E. D., Mushinsky, H. R., Richards, C. L., & Martin, L. B. (2012). Epigenetic Variation May Compensate for Decreased Genetic Variation with Introductions: A Case Study Using House Sparrows (*Passer domesticus*) on Two Continents. *Genetics Research International*, 2012(2090–3154), 979751. https://doi.org/10.1155/2012/979751

Schwartz, M. K., Luikart, G., & Waples, R. S. (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution*, 22(1), 25–33. https://doi.org/10.1016/j.tree.2006.08.009

Sendell-Price, A. T., Ruegg, K. C., & Clegg, S. M. (2020). Rapid morphological divergence following a human-mediated introduction: the role of drift and directional selection. *Heredity*, 124(4), 535–549. https://doi.org/10.1038/s41437-020-0298-8

Siwertsson, A., Knudsen, R., Adams, C. E., Præbel, K., & Amundsen, P. A. (2013). Parallel and non-parallel morphological divergence among foraging specialists in European whitefish (*Coregonus lavaretus*). *Ecology and Evolution*, 3(6), 1590–1602. https://doi.org/10.1002/ece3.562

Smith, G., Smith, C., Kenny, J. G., Chaudhuri, R. R., & Ritchie, M. G. (2015). Genome-wide DNA methylation patterns in wild samples of two morphotypes of threespine stickleback (*Gasterosteus aculeatus*). *Molecular Biology and Evolution*, 32(4), 888–895. https://doi.org/10.1093/molbev/msu344

Smith, T. A., Martin, M. D., Nguyen, M., & Mendelson, T. C. (2016). Epigenetic divergence as a potential first step in darter speciation. *Molecular Ecology*, 25(8), 1883–1894. https://doi.org/10.1111/mec.13561

This article is protected by copyright. All rights reserved
Smithson, M. W., Dybdahl, M. F., & Nuismer, S. L. (2019). The adaptive value of epigenetic mutation: limited in large but high in small peripheral populations. *Journal of Evolutionary Biology*, jeb.13535. https://doi.org/10.1111/jeb.13535

Song, L., Zhang, J., Li, C., Yao, J., Jiang, C., Li, Y., Liu, S., & Liu, Z. (2014). Genome-Wide Identification of Hsp40 Genes in Channel Catfish and Their Regulated Expression after Bacterial Infection. *PLoS ONE*, 9(12), e115752. https://doi.org/10.1371/journal.pone.0115752

Stacklies, W., Redestig, H., Scholz, M., Walther, D., & Selbig, J. (2007). pcaMethods - A bioconductor package providing PCA methods for incomplete data. *Bioinformatics*, 23(9), 1164–1167. https://doi.org/10.1093/bioinformatics/btm069

Stajic, D., Perfeito, L., & Jansen, L. E. T. (2019). Epigenetic gene silencing alters the mechanisms and rate of evolutionary adaptation. *Nature Ecology and Evolution*, 3(3), 491–498. https://doi.org/10.1038/s41559-018-0781-2

Swan, K. D., Lloyd, N. A., & Moehrenschlager, A. (2018). Projecting further increases in conservation translocations: A Canadian case study. *Biological Conservation*, 228, 175–182. https://doi.org/10.1016/j.biocon.2018.10.026

Syväranta, J., & Jones, R. I. (2008). Changes in feeding niche widths of perch and roach following biomanipulation, revealed by stable isotope analysis. *Freshwater Biology*, 53(3), 425–434. https://doi.org/10.1111/j.1365-2427.2007.01905.x

Szűcs, M., Melbourne, B. A., Tuff, T., Weiss-Lehman, C., & Hufbauer, R. A. (2017). Genetic and demographic founder effects have long-term fitness consequences for colonising populations. *Ecology Letters*, 20(4), 436–444. https://doi.org/10.1111/ele.12743

Taylor, H. R. (2015). The use and abuse of genetic marker-based estimates of relatedness and inbreeding. *Ecology and Evolution*, 5(15), 3140–3150. https://doi.org/10.1002/ece3.1541

Terekhanova, N. V., Logacheva, M. D., Penin, A. A., Neretina, T. V., Barmintseva, A. E., Bazykin, G. A., Kondrashov, A. S., & Mugue, N. S. (2014). Fast Evolution from Precast

This article is protected by copyright. All rights reserved
Bricks: Genomics of Young Freshwater Populations of Threespine Stickleback Gasterosteus aculeatus. *PLoS Genetics*, 10(10), e1004696. https://doi.org/10.1371/journal.pgen.1004696

Thomas, P. D., Campbell, M. J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R., Diemer, K., Muruganujan, A., & Narechania, A. (2003). PANTHER: A library of protein families and subfamilies indexed by function. *Genome Research*, 13(9), 2129–2141. https://doi.org/10.1101/gr.772403

van der Graaf, A., Wardenaara, R., Neumann, D. A., Taudt, A., Shaw, R. G., Jansen, R. C., Schmitz, R. J., Colomé-Tatché, M., & Johannes, F. (2015). Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *Proceedings of the National Academy of Sciences of the United States of America*, 112(21), 6676–6681. https://doi.org/10.1073/pnas.1424254112

Vander Zanden, M. J., Clayton, M. K., Moody, E. K., Solomon, C. T., & Weidel, B. C. (2015). Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis. *PLOS ONE*, 10(1), e0116182. https://doi.org/10.1371/journal.pone.0116182

Vatsiou, A. I., Bazin, E., & Gaggiotti, O. E. (2016). Changes in selective pressures associated with human population expansion may explain metabolic and immune related pathways enriched for signatures of positive selection. *BMC Genomics*, 17(1), 1–11. https://doi.org/10.1186/s12864-016-2783-2

Vincent, B., Dionne, M., Kent, M. P., Lien, S., & Bernatchez, L. (2013). Landscape genomics in atlantic salmon (*Salmo salar*): Searching for gene-environment interactions driving local adaptation. *Evolution*, 67(12), 3469–3487. https://doi.org/10.1111/evo.12139

Wang, M., Zhao, Y., & Zhang, B. (2015). Efficient Test and Visualization of Multi-Set Intersections. *Scientific Reports*, 5(1), 16923. https://doi.org/10.1038/srep16923

Waters, C. D., Hard, J. J., Fast, D. E., Knudsen, C. M., Bosch, W. J., & Naish, K. A. (2020). Genomic and phenotypic effects of inbreeding across two different hatchery management regimes in Chinook salmon. *Molecular Ecology*, 29(4), 658–672. https://doi.org/10.1111/mec.15356
Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution, 38*(6), 1358–1370.

Willoughby, J. R., Harder, A. M., Tennessen, J. A., Scribner, K. T., & Christie, M. R. (2018). Rapid genetic adaptation to a novel environment despite a genome-wide reduction in genetic diversity. *Molecular Ecology, 27*(20), 4041–4051. https://doi.org/10.1111/mec.14726

Yohannes, E., Grimm, C., Rothhaupt, K.-O., & Behrmann-Godel, J. (2017). The Effect of Parasite Infection on Stable Isotope Turnover Rates of δ15N, δ13C and δ34S in Multiple Tissues of Eurasian Perch *Perca fluviatilis*. *PLOS ONE, 12*(1), e0169058. https://doi.org/10.1371/journal.pone.0169058

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics, 28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606
Tables

Table 1 List of populations sampled, the system they belong to, whether they are source or refuge, the year refuge populations were established and the life stage of translocated individuals, expected ($H_E$) and observed ($H_O$) heterozygosity, and nucleotide diversity ($\pi$). Significant differences in heterozygosity and nucleotide diversity in refuge populations from the source are denoted by *.

| Lake       | System | Lake type | Year of refuge translocation | Life stage introduced | $H_E$ | $H_O$ | $\pi$ |
|------------|--------|-----------|-------------------------------|-----------------------|-------|-------|-------|
| Eck        | Eck    | Source    |                               |                       | 0.345 | 0.361 | 0.00378 |
| Glashan    | Eck    | Refuge    | 2010-2011                     | fry, adults           | 0.331* | 0.352* | 0.00369 |
| Tarsan     | Eck    | Refuge    | 2010-2011                     | fry, adults           | 0.334* | 0.350* | 0.00374 |
| Lomond     | Lomond | Source    |                               |                       | 0.329 | 0.351 | 0.00366 |
| Allt na Lairige | Lomond | Refuge    | 2009-2010                     | eggs, fry             | 0.305* | 0.335* | 0.00359* |
| Shira      | Lomond | Refuge    | 2009-2010                     | eggs, fry             | 0.319* | 0.337* | 0.00365 |
| Carron Valley | Lomond | Refuge    | 1988-1990                     | fry                   | 0.308* | 0.327* | 0.00351* |
| Sloy       | Lomond | Refuge    | 1988-1990                     | fry, adults           | 0.309* | 0.326* | 0.00347* |
Table 2 Test statistic, p-value, and the direction of the significant differences from the Kolmogorov-Smirnov tests for $F_{H}$ and $R_{xy}$ indices. Tests were conducted between source (S) and refuge (R) populations for the Eck and Lomond systems.

| Comparison | Lakes       | $R_{xy}$ | p-value | Difference | $F_{H}$ | p-value | Difference |
|------------|-------------|----------|---------|------------|---------|---------|------------|
| Eck system | Eck vs Glashan | 0.74     | < 0.001 | R > S      | 0.32    | 0.14    | NA         |
|            | Eck vs Tarsan | 0.72     | < 0.001 | R > S      | 0.39    | 0.04    | R > S      |
| Lomond system | Lomond vs Allt na Lairige | 0.66     | < 0.001 | R > S      | 0.69    | < 0.001 | R > S      |
|            | Lomond vs Shira | 0.82     | < 0.001 | R > S      | 0.72    | < 0.001 | R > S      |
|            | Lomond vs Carron | 0.99     | < 0.001 | R > S      | 0.69    | < 0.001 | R > S      |
|            | Lomond vs Sloy | 0.99     | < 0.001 | R > S      | 0.82    | < 0.001 | R > S      |
Table 3 Pairwise Weir and Cockerham $F_{ST}$ between each population for the Eck and Lomond systems. Bold values indicate significant differentiation.

| Eck system | Eck    | Glashan | Tarsan |
|------------|--------|---------|--------|
| Eck        | -      |         |        |
| Glashan    | 0.003  | -       |        |
| Tarsan     | 0.002  | 0.0001  | -      |

| Lomond system | Lomond | Allt na Lairige | Shira | Carron Valley | Sloy |
|---------------|--------|-----------------|-------|---------------|-----|
| Lomond        | -      |                 |       |               |     |
| Allt na Lairige | 0.006 | -               |       |               |     |
| Shira         | 0.004  | 0.003           | -     |               |     |
| Carron Valley | 0.020  | 0.030           | 0.026 | -             |     |
| Sloy          | 0.030  | 0.041           | 0.032 | 0.050         | -   |
Figure Legends

Figure 1. Map indicating the location of the source and refuge populations of European whitefish in Scotland, with a simplified representative fish shown. Populations from the Eck system are represented by circles, populations from the Lomond system by triangles. Source populations are in grey, refuge populations in colour.

Figure 2. Morphological and stable isotope analyses. a) Principal component analysis for all populations. Arrows indicate direction of body shape change from source to refuge populations. Body shape differences between highest and lowest values of PC1 and PC2 are reported on each axis. Points represent mean value for each population, with bars showing standard error of the mean. b) Convex hull area of the scaled stable isotopes for muscle tissue, which are described by the IRic index.

Figure 3. Population genomic analyses. a) Principal component analysis of the full genomic dataset displaying PC1 and PC2. b) Distribution of pairwise relatedness (Rxy) and inbreeding coefficient (F_H) indices for the Eck and Lomond system datasets. Significant differences were observed for all comparisons between source and refuge, except for Glashan which did not differ in F_H from the Eck population.

Figure 4. Genomic outliers of translocation. a) Redundancy analysis (RDA) of the combined genomic dataset, using lake system (97% of variation, RDA 1) and lake type (3% of variation, RDA 2) as response variables. b) Number of outlier SNPs identified by RDA (70, blue), BayPass (21, yellow) and the SNPs that overlap (14, red). The gene IDs correspond to the shared outlier genes between RDA and BayPass. c) Distribution of RDA (blue), BayPass (yellow), and shared (red) outlier SNPs along the genome. The y axis represents the absolute allele frequency change (z-score) between source and refuge populations. Chromosomes are coloured alternating black and grey.

Figure 5. Results of the epigenomic analyses. a) Number of differentially methylated loci between source and refuge populations in the Lomond system shared across comparisons. b) Number of differentially methylated loci between source and refuge populations in the Eck system shared across comparisons. c) Redundancy analysis (RDA) of the combined epigenomic dataset, with lake system (65% of variation) and lake type (35% of variation) as response variables. d) Heatmap of the normalised log_2 transformed 1,493 loci identified by the RDA as associated with lake type. Rows represent the loci, and columns represent
individuals. Locus Z-score represents the number of standard deviations of away from the mean of the log transformed read counts in the dataset for each sample.

Figure 6. Proportion of morphological variation explained by genomic and epigenomic variation calculated using RDAs. Each panel of the variation partitioning decomposes the morphological variance in the combined, Eck, Lomond, and young populations (7-9 years since translocation) datasets, and Lomond and old populations (30 year since translocation) dataset. The total amount of morphological variance explained by the data corresponds to the ‘Genomic & Epigenomic’ category, while the remaining part is associated to the statistically non-testable (NT) ‘Residuals’. The proportion of variance associated to ‘Genomic’ (white), ‘Epigenomic’ (light blue) variation and their intersection (middle) are decomposed in the Venn diagrams. Significant effects are noted by the *.
Figure (a) shows a scatter plot with principal component analysis (PCA) for different lakes: Lake Glashan, Lake Tarsan, Lake Eck, Allt na lairige, Lake Shira, Lake Carron, Lake Sloy, and Lake Lomond. The PC1 axis represents 22% of the variance, and the PC2 axis represents 20% of the variance.

Figure (b) displays the scatter plot with scaled δ¹³C values. The axes range from -0.012 to 0.012 for PC1 and from 0 to 1 for scaled δ¹³C. The data points for each lake are represented by different colors and shapes, indicating their positions in the PCA space.
Figure (a) shows a principal component analysis (PCA) plot with two distinct clusters, labelled as "Source population" (orange circles) and "Refuge population" (green triangles). The plot is divided by the Eck and Lomond system, and the X-axis represents the first RDA (97%).

Figure (b) features a Venn diagram illustrating the overlap of gene expression changes between RDA and BayPass. The gene names DJC18, LADD, GRIN3, AT8B1, and TLR13 are mentioned, with the number of overlapping genes indicated as 70, 14, and 21, respectively.

Figure (c) displays a scatter plot titled "Absolute allele frequency change (z-score)" against the chromosome number. The plot includes a range from 0.0 to 5.0 on the y-axis and from 1 to 40 on the x-axis, showing a distribution of allele frequency changes across different chromosomes.
Combined dataset

Genomic & Epigenomic: 18% ***
Genomic: 7% ***
Epigenomic: 16% ***

Residuals: 82% NT

Eck dataset

Genomic & Epigenomic: 29% ***
Genomic: 1%
Epigenomic: 27% ***

Residuals: 71% NT

Lomond dataset with young populations

Genomic & Epigenomic: 0.3%
Genomic: 2%
Epigenomic: 1%

Residuals: 99% NT

Lomond dataset with old populations

Genomic & Epigenomic: 16% *
Genomic: 13% *
Epigenomic: 6%

Residuals: 84% NT