Adams, C.E.; Bean, C.W.; Dodd, J.A.; Down, A.; Etheridge, E.C.; Gowans, A.R.D.; Hooker, O.; Knudsen, R.; Lyle, A.A.; Winfield, I.J.; Praebel, K. 2016.
Inter and intra-population phenotypic and genotypic structuring in the European whitefish Coregonus lavaretus, a rare freshwater fish in Scotland. *Journal of Fish Biology*, 88 (2). 580-594. 10.1111/jfb.12855

Inter and intra-population phenotypic and genotypic structuring in the European whitefish, *Coregonus lavaretus*, a rare freshwater fish in Scotland.

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Key words: early divergence, ecological evolution, speciation

Running head: Phenotypic and genetic structuring in powan

ABSTRACT

Intra-specific structuring of phenotype and genotype provides an insight into the evolutionary processes that have shaped the species. This study revealed between-lake genetic structuring between *Coregonus lavaretus* collected from the only 2 native

populations of this species in Scotland (Lochs Eck and Lomond) evidenced by the existence of private alleles (12 in Lomond and 4 in Eck) and significant genetic differentiation (F_{ST}=0.056) across 10 microsatellite markers. Juvenile *C. lavaretus* originating from eggs collected from the two lakes and reared in a common garden experiment showed clear phenotypic differences in trophic morphology (i.e., head and body shape) between these populations indicating that these characteristics were, at least partly, inherited. Microsatellite analysis of adults collected from different geographic regions within Loch Lomond revealed detectable and statistically significant but relatively weak genetic structuring (F_{ST} = 0.001 - 0.024) and evidence of private alleles related to the basin structure of the lake. Within-lake genetic divergence patterns suggest three possibilities for this observed pattern i) differential selection pressures causing divergence into separate gene pools ii) a collapse of two formerly divergent gene-pools iii) a stable state maintained by balancing selection forces resulting from spatial variation in selection and lake heterogeneity. Small estimates of effective population sizes for the populations in both lakes suggest that the capacity of both populations to adapt to future environmental change may be limited.

INTRODUCTION

There is increasing evidence of intraspecific structuring of both phenotype and genotype over multiple spatial scales for species from a broad range of taxonomic groups (as exemplars see (Kang *et al.,* 2013) on crustaceans; (Lozier, 2013) on insects; (Jorde *et al.,* 2007; Fevolden *et al.,* 2012) on fish; (Piertney *et al.,* 1998) on birds and (Swislocka *et al.* 2013) on mammals.

It is generally assumed, but only rarely directly tested, that phenotypic structuring reflects localised evolutionary responses resulting from exposure to differing environments across the range of the species. This operating either through differential selection pressures shaping local adaptation (Garant *et al.,* 2007) or imposing localised phenotypic change through plasticity responses during ontogeny (Adams & Huntingford, 2004). Although intra-specific genetic structuring might be reasonably assumed

to be a local adaptive response reflecting differential selection pressures (Hendry & Stearns, 2004), it could also be the result of non-directional genome change such as random genetic drift or population bottlenecking (Frazer & Rusello, 2013).

The context within which either, or both, of phenotypic or genetic structuring within a species might occur is significantly influenced by the patterns of dispersal and gene flow across the species. For example intraspecific phenotypic or genetic structuring resulting from differential selection pressures across the range of the species is likely to be limited in extent by unrestricted gene flow. Phenotypic variation resulting purely from plastic responses to the environment may however, endure in such panmictic populations. Thus high levels of intra-specific structuring are more likely in species which are disjunct or fragmented in distribution across their range.

Freshwater systems comprise a series of geographically disjunct habitats. Moreover some obligate freshwater-living fishes have generally limited powers of dispersal (Adams & Maitland 2001) and thus may be reasonably expected to exhibit intraspecific structuring across systems. There is strong evidence that this does occur, for example in Arctic charr *Salvelinus alpinus* (L. 1758) (Wilson *et al.,* 2004; Bush & Adams, 2007; Garduño-Paz *et al.,* 2012)), brown trout *Salmo trutta* L.1758 (Taggart *et al.,* 1981; Ferguson, 1989), and even among anadromous populations of Atlantic salmon *Salmo salar* L.1758 (Jordan *et al.,* 1992; King *et al.,* 2001; King, 2007; Landry & Bernatchez, 2001).

In addition to between-catchment phenotypic and genetic structuring, there is now a considerable body of evidence of structuring in populations within a single catchment. Examples include North American lake whitefish *Coregonus clupeaformis* (Mitchill 1818) (Gagnaire *et al.,* 2013); pygmy whitefish *Prosopium coulterii* (Eigenmann & Eigenmann, 1892) (Gowell *et al.,* 2012); European whitefish *Coregonus lavaretus* (L.1758) (Præbel *et al.* 2013a; Siwertsson *et al.* 2013; Dierking *et al.* 2014); three spined sticklebacks *Gasterosteus aculeatus* L. 1758 (Defaveri *et al.,* 2013); nine spined stickleback *Pungitius pungitius* (L. 1758) (Ishikawa *et al.,* 2013) and Arctic charr (Danzmann *et al.,* 1991; Adams *et al.,* 1998; Fraser *et al.,*1998; Knudsen *et al.,* 2007). At least for

some species, the geographic scale of the habitat seems to be an important factor influencing phenotypic and genetic structuring (Chavarie *et al.* 2014).

In many places, particularly in post-glacial freshwater systems, observed structuring within a single system is known to have arisen recently within the catchment (Gislason *et al.,* 1999; Garduño-Paz *et al.,* 2012). There is considerable evidence that much of the structuring of phenotype is to be found in traits that have functional significance related to the capture and consumption of prey (trophic morphology *sensu* (Skulason & Smith 1995; Schluter 2009). There are several well studied examples where the phenotypic structuring of a species in a single catchment takes the form of well-defined, phenotypically discrete, morphological groups, that also exhibit clear ecological differences and which comprise separate gene pools with limited, or no, gene flow but living in apparent sympatry (exemplars include Arctic charr (Snorrason *et al.,* 1994; Adams *et al.,* 1998; Klemetsen *et al.,* 2006) three-spined stickleback (Olafsdóttir *et al.,* 2006) and European whitefish (Siwertsson *et al.,* 2012). Elsewhere within-species structuring in a single ecosystem is apparently more subtle (Svanbäck & Eklöv, 2004; Hendry *et al.,* 2009;(Hirsch *et al.* 2013) and at least in some circumstances, it may be reversible (Taylor *et al.,* 2006; Bittner *et al.,* 2010; Vonlanthen *et al.,* 2012; Bhat *et al.,* 2014).

Where intra-specific, genotype and /or phenotype structuring does occur, the patterns of its organisation can be highly informative. Structuring is indicative of the very early stages of the diversifying evolutionary processes that may ultimately lead to new species. As a result, structuring patterns can indicate the contemporary diversifying selection pressures to which a species is exposed, providing insights into the mechanisms of biodiversity formation (Urban *et al.,* 2008). More practically, such structuring is an indicator of the adaptive potential of natural populations to respond to environmental change (Bolnick *et al.,* 2011). For species for which biodiversity conservation or fisheries management is required, the existence of structuring is likely to have significant implications for the identification of useful management units (Rader *et al.,* 2005). Thus genotypic or phenotypic structuring in organisms of high conservation value may be particularly important.

Here we examine population structuring in one of the fishes considered above, *Coregonus lavaretus* (known in Scotland as powan), a lacustrine species of high conservation and fisheries value in many parts of Europe. The study was structured over two spatial scales; between two lakes in two adjacent but unconnected catchments and between basins within a single lake. The study tests the hypothesis that a) structuring will be greater across lakes than within a lake and b) that local evolutionary responses to exposure to differential environments should result in significant genetic structure between sites in the larger lake (Loch Lomond).

METHODS

STUDY SITE

Coregonus lavaretus is found naturally in only two lakes in Scotland and seven in the UK as a whole. The largest of these is Loch Lomond, (56° 7' 13.9" N 004° 37' 45.3" W) a glacially formed freshwater lake of 71 km^2 and 198 m maximum depth, which comprises three distinct basins. The species feeds predominantly on zooplankton in the pelagic zone in Lomond (Pomeroy, 1991) and are commonly found throughout the lake. Loch Eck (56° 4' 44.2" N; 004° 59' 45.7" W) is similarly glacial in origin although smaller (4.4 km^2; 42 m maximum depth) and, although only 20 km from Loch Lomond, these two lakes are not part of the same catchment, having independent drainages to the sea (Fig.1).

COMMON GARDEN EXPERIMENT

Male and female sexually mature *C. lavaretus* were collected under licence (Scottish Natural Heritage licence # 9242) from both Loch Lomond from sites 6,7 and 8 and Loch Eck (site 1) in January 2010 (Table I). Eggs were stripped from ovulating females, split into 2 batches and each fertilised with milt from a single male. 100 families comprising the eggs of around 50 females were created from fish collected from Loch Eck and 46 families (23 females) from the mid basin of Loch Lomond. Eggs were incubated in

12 vertical incubation bottles (Rottman & Shireman, 1988). On hatching the alevins were transferred to 10, 20L tanks comprising mixed families but separate lake of origin and at first feeding were fed a small particle size commercial diet. At 13 days after their first feeding, 30 individuals were taken randomly from tanks containing juveniles from Loch Eck and Loch Lomond, anaesthetised and photographed in left, lateral view on a scale.

Landmark-based geometric morphometric analysis was used to detect variation in the body shape of individual fish. Eleven landmarks (Fig. 2) were located and digitized (using tpsDig2; Rohlf, 2006). Generalized least squares Procrustes superimposition was performed using the program “Morpho-J” (Klingenberg, 2011) to rotate, scale and translate landmarks, and produce Procrustes coordinates. To compare shape differences between groups “Morpho-J” was also used to conduct a multivariate Discriminant Function Analysis of Procrustes coordinates (Zelditch *et al.,* 2004; Klingenberg, 2011; Garduño-Paz *et al.,* 2012).

POPULATION GENETIC ANALYSIS

Coregonus lavaretus were collected for genetic analysis by gill net from across Loch Eck’s single basin and from each of Loch Lomond’s three basins around the breeding season from between November and January between 2006 and 2009. Norden survey gill nets comprising 12 panels, ranging from 5 to 55 mm, knot-to-knot mesh were set on the lake bottom overnight at 4 sites in the north (subsequently “Lomond-N”), 2 site in the south (“Lomond-S”) and 3 in the mid basins (“Lomond-M”) of Loch Lomond (Fig. 1; Table I) and at multiple sites in Loch Eck adjacent to sites 1 and 2 (subsequently “Eck”) (Fig 1; Table I). Fish were captured in water depths ranging from 2 to 15m. Fish were removed and an adipose fin clip removed and stored in 100% ethanol. As the samples collected from fish in the mid and south basin of Loch Lomond could not be genetically discriminated and the pooled sample was within Hardy-Weinberg equilibrium (Table II) samples from these sites were pooled to compare between lakes to increase statistical power.

Genomic DNA was extracted from fin clips using E-Z96 Tissue DNA Kit (OMEGA Bio-tek) following the manufacturer's instructions. A total of 15 microsatellite loci; BFRO-018 (Susnik, Snoj & Dovc 1999) BWF1, BWF2 (Patton *et al.,* 1997), C2-157 (Turgon et al., 1999) Cla-Tet01, Cla-Tet03, Cla-Tet10, Cla-Tet13, Cla-Tet15, Cla-Tet18 (Winkler & Weiss, 2008a) Cocl-lav04, Cocl-lav06, Cocl-lav10, Cocl-lav18, and Cocl-lav49 (Rogers *et al.,* 2004) (Table S1) were amplified using forward-labelled primers in four PCR multiplexes following the protocol by (Præbel *et al.,* 2013b). The PCR products were separated on an ABI 3130 XL Automated Genetic Analyser (Applied Biosystems) using GENESCAN LIZ-500 (Applied Biosystems) as a size standard. The binning and scoring was performed in GENEMAPPER 3.7 (Applied Biosystems) and manually verified. Replicate (5-9 %) and blind (4 %) samples were included in all PCR's to confirm consistency of scoring and absence of contamination. The repeatability and consistency of genotypes were 100 %. The samples were screened for abnormalities in the software MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004), using 1,000 bootstraps to generate the expected homozygote and heterozygote allele size difference frequencies.

Allelic richness (N_{AR}) and private allelic richness (N_{PAR}) were determined for fish grouped by sampling area (3 sites within Loch Lomond and between Loch Lomond (mid and south basins pooled) and Loch Eck). Differences in sample sizes were accounted for using the rarefaction procedure for the smallest sample size (80 genes) as implemented in the software HP-RARE 1.0 (Kalinowski, 2005). In the intralacustrine analysis of Loch Lomond, N_{AR} and N_{PAR} were normalised for differences in sample sizes using 36 genes and the pair-wise occurrence of private alleles between the three sampled basins were also estimated. Expected heterozygosity (H_e) and Wrights F_{IS} across loci and departure from the Hardy-Weinberg equilibrium (HWE) were tested by exact tests (Guo & Thompson, 1992) as implemented in GENEPOP 4.0 (Rousset, 2007). A test for the Wallund effect,

indicating any structuring within Loch Lomond was conducted on pooled samples which were tested for deviations from Hardy-Weinberg expectations. Samples were also tested for significant association with a heterozygote deficit or excess by the exact test implemented in GENEPOP 4.0. Pair-wise comparisons were corrected for multiple comparisons using sequential Bonferroni corrections following Rice (Rice *et al.,* 2008). Allelic richness and the relative frequency of private alleles were compared using a paired-t test under no *a priori* assumption of the direction of any difference between pairs. Standard genetic diversity measures; number of alleles (N_A), H_e, and observed heterozygosity (H_o) for each locus per population were estimated in GenAlEx (Peakall & Smouse, 2006).

Genetic differentiation between lakes and between basins within Loch Lomond were estimated by F_{ST} and tested for significance by 10,000 permutations using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010). Additionally, F_{ST} per locus per population was estimated using GENEPOP 4.0 (Rousset, 2007) for a *post hoc* evaluation of the contribution of each locus to the observed genetic pattern.

The effective population size (N_e) was also estimated for Loch Lomond and Loch Eck populations using OneSamp 1.1 (Tallmon *et al.,* 2008) which uses a Bayesian computation to estimate N_e from summary statistics that are related to N_e (see Tallmon *et al.,* 2008 for details). As recommended in the program manual, a wide range of prior upper and lower bounds for N_e (2-1000) was initially tested in several replicas and ranges to identify the prior resulting in the narrowest 95% credible intervals. Prior upper and lower bounds for N_e of 20-200 and 10,000 replications were used to generate the 95% credible intervals. The analyses were run in triplicate to ensure consistency of the estimate.

RESULTS

COMMON GARDEN EXPERIMENT

Discriminant Function Analysis clearly segregated juvenile post-first feeding, *C. lavaretus* from Loch Lomond from those at the same developmental stage from Loch Eck on the basis of shape when reared in the laboratory under identical conditions (Fig. 3). Both the mean Procrustes coordinate difference and the Mahalanobis distance were significantly different between individuals originating from the two lakes (Procrustes distance = 0.010; $P<0.001$; Mahalanobis distance = 1.81 $P<0.002$). Discriminant Function Analysis correctly assigned, on the basis of shape only, 71.9% of Loch Eck origin juveniles and 81.1% of Loch Lomond juveniles, to their lake of origin. Loch Eck juveniles had a rounder, more robust head and deeper body compared with those from Loch Lomond.

VALIDATION AND QUALITY CONTROL OF GENOTYPIC DATA

The loci BFRO-018, BWF2, Cocl-lav04, and Cocl-lav10 were monomorphic in all individuals and were therefore omitted from the analysed dataset. The locus Cocl-lav49 was monomorphic in Loch Eck and was therefore excluded from the interlacustrine comparisons. Heterozygote deficits were indicated by MICRO-CHECKER only at the ClaTet18 locus for individuals from Lomond-N, Lomond-S and for Lomond-S+M. The heterozygote deficits were associated with more than 50 % of the alleles being in one allele class and were suggestive of the presence of null alleles. None of the samples showed significant departures from HWE after sequential Bonferroni corrections (Table S1). Given the relatively few loci used in the study, ClaTet18 was therefore retained in the analysis to ensure statistical power. Thus, the following analyses are based on 10 and 11 loci for the inter-lacustrine (Lomond-M+S vs. Eck) and intra-lacustrine (Lomond-N, Lomond-S Lomond-M) comparisons, respectively.

GENETIC DIVERSITY AND DIVERGENCE BETWEEN LOCH LOMOND AND LOCH ECK

The number of alleles per locus ranged from 2 to 8 across the 10 loci used in this study (Table S1). There was no evidence of a global departure from HWE (P = 0.832) for any of the samples (Table II). Loch Lomond displayed slightly higher mean allelic richness (Lomond-S+M, N_{AR} = 4.7±0.6; mean±SE) than Loch Eck (vs. Eck N_{AR} = 3.8±0.5) (paired t-test; P<0.01) across all loci. In addition, total private allelic richness was higher for Loch Lomond fish (Lomond-S+M, N_{TPAR} = 12) than for Loch Eck (N_{TPAR} = 4,) and mean private allelic richness was statistically greater for Lomond (Lomond-S+M N_{PAR} = 1.2±0.3; mean ±SE) than for Loch Eck (Eck N_{PAR} = 0.4±0.1) (P =0.023). The expected heterozygosity was similar between the two populations (Table II) (H_e = 0.48 for Lomond-S+M and 0.42 Eck) (P =0.47).

Between lake genetic structuring was further evidenced by a highly significant (P <0.0001) pair-wise genetic difference across all loci (F_{ST} = 0.056) between Loch Lomond and Loch Eck.

The effective population size estimates did not differ significantly between Loch Lomond (Ne = 54, CI 45-72) and Loch Eck (Ne = 52, CL= 43-70) (Table II).

GENETIC DIVERSITY AND DIVERGENCE WITHIN LOCH LOMOND

The number of alleles per locus was generally low, ranging from 2 to 8 across the 11 loci examined in Loch Lomond fish (Table S2). An estimate of deviations from HWE of all groups combined (F_{IS} = 0.0109; P <0.001) showed departure from HWE

associated with a heterozygote deficit, indicating a Wahlund effect or the general presence of null alleles. In contrast none of the individual sampled groups showed significant departures from the HWE-expectations (Table II).

Mean overall allelic richness when measured across all loci did not differ significantly between sites in Loch Lomond (Lomond-N N_{AR} = 4.1; Lomond-M N_{AR} = 3.6; Lomond-S N_{AR} = 3.9;) (F=0.25; P =0.776) (Table II). Eight loci showed evidence of private alleles (N=20) in fish from at least one sampling site in Loch Lomond (BWF1, ClaTet03, ClaTet13, ClaTet18, ClaTet10, ClaTet15, C2-157 and ClaTet01). Mean private allelic richness across these loci (i.e. alleles that are unique to fish from that basin) differed significantly between individuals from the north and mid basins (Lomond- N N_{PAR} = 0.42; Lomond-M N_{PAR} = 0.25; P <0.03), between individuals from the north and south basins (Lomond-S N_{PAR} = 0.38; P <0.006) and between mid and south basins (P <0.003) (Table II). However, none of the private alleles showed frequencies higher than (5.6 %) when comparing among the three samples (Table S1).

A pairwise comparison of north basin and mid basin samples using all 11 microsatellite loci showed a significant F_{ST} value (LLN and LLM: F_{ST} = 0.020, P = 0.021). In contrast, it was not possible to discriminate between the north and south basin samples (LLN & LLS; F_{ST} = -0.001, P = 0.511), nor between south and mid basin samples (LLS and LLM; F_{ST} = 0.004, P = 0.246). Where significant, the pairwise F_{ST} values are still below the threshold where an individual based group clustering analysis (such as STRUCTURE) is appropriate for allocating indi~~c~~viduals to specific group~~s~~ings (Prichard *et al.* 2007), indicating significant but very weak within-lake structuring.

DISCUSSION

This study demonstrates genetic and phenotypic structuring across native populations of European whitefish from two discrete water-courses, separated by ca 20km, as well as within lake differences in the larger Loch Lomond. Between-lake genetic structuring is evidenced by the existence of private alleles (12 in Lomond and 4 in Eck) and significant genetic differentiation (F_{ST}) across the microsatellite loci used here. The magnitude of this genetic differentiation (F_{ST} = 0.056) appears intermediate compared with that found between populations of this species isolated from each other elsewhere. For example, the genetic differentiation between two northern Norwegian lake populations with an F_{ST} = 0.16 is three times higher than that found here (Præbel *et al.* 2013a). Whereas comparison with several Finnish and German populations revealed inter-population genetic differentiation in the range from F_{ST} < 0.01 to 0.21 (Saisa *et al.,* 2008; Dierking *et al.,* 2014).

There were correspondingly clear phenotypic differences between the F1 progeny of fish from the two lakes in the common garden experiment presented here. Etheridge and her co-workers (Etheridge *et al.* 2012) showed clear and significant differences in functional, phenotypic traits (the shape of the head and mouth; foraging morphology *sensu* Skulason *et al.,* 1999) between adult *C. lavaretus* from Loch Eck and Loch Lomond. However it was not possible to determine if these phenotypic differences were inherited across generations or were the result of differential expression of plastic traits (Adams & Huntingford, 2002, 2004). The common garden experiment reported here shows that at least some of the expressed variation in head- and body morphology is inherited. Head-shape variation is commonly strongly related to the different foraging niches in polymorphic populations of postglacial freshwater fish (Adams *et al.* 1998; Fraser *et al.* 1998; Siwertsson *et al.* 2013). The variation described here in juveniles (a rounder more robust head and deeper body in Loch Eck fish) is similar to the intraspecific morphological differences described for plankton and macrobenthos foraging specialists of other species (Skulason *et al.* 1994; Adams *et al.* 1998; Adams and Huntingford 2002). This is mirrored in differences between populations in foraging ecology, with Loch Lomond fish feeding

predominantly on plankton and the Loch Eck fish preying primarily on macrobenthos (Pomeroy, 1991) and parasitic loading (Etheridge *et al.* 2012) between these two populations. It is most likely that these trophic related traits are inherited genetically, although the possibility of a maternally-induced effect (for example through differential egg provisioning) cannot be completely ruled out. It is probable that the populations of *C. lavaretus* in Loch Eck and Loch Lomond were established at about the same time by anadromous *Coregonus* ancestors of the current populations, following the retreat of the glaciers around 12,000 years ago (Clark *et al.,* 2012). Given the close geographic proximity of these populations to each other and the common sea route to invasion of the emerging fresh waters, a logical and parsimonious assumption is that both locations were invaded by a single common ancestor. The relatively modest magnitude of the genetic divergence at variable, but neutral, genetic markers used here lends additional support to this interpretation. If that is the case, then the contemporary between-lake, genetic and phenotypic pattern indicates that, in the approximately 4000 generations since invasion, these two populations have diverged markedly genetically and phenotypically, including in functional traits related to foraging and that they have a genetic basis to their expression (see also Etheridge *et al.,* 2012).

This study also shows subtle, but detectable, genetic structuring within the larger of the habitats supporting the species examined here. Although analysis of the spatial patterning of this genetic structuring in this current study is relatively crude, it maps on to physical structuring of the lake. Detectible and significant population differentiation between fish caught during the breeding period in each of the three principal lake basins were identified. Using all 11 microsatellite loci, F_{ST} analysis indicates clear genetic differences between *C. lavaretus* from the north and mid basins but not between south and mid basins. This structuring is also supported by the occurrence of private alleles where fish from the geographically most distant sampling locations (north and south Lomond basins) display a higher number of private alleles than the intermediate sampling locality (the mid basin of Lomond). Since private alleles generally occur in low frequency in recently diverged populations they do not contribute significantly to the estimates of genetic differentiation (e.g. F_{ST}) and therefore provide an alternative measure of genetic isolation (Szpiech & Rosenberg, 2011).

Siwertsson and colleagues (Siwertsson *et al.,* 2013), found genetic differences between *Coregonus lavaretus* ecomorphs, using the same microsatellite loci used in this study, which were also clearly defined by ecological, morphological and meristic characters in lakes, in a similar range (F_{ST} 0.014 to 0.024) as those reported here. The subtle nature of the between-basin genetic structuring amongst the *C. lavaretus* from Loch Lomond reported here indicates that the assortative mating that has resulted in this structuring, is either a relatively new phenomenon or that there remains some considerable gene flow between fish in different basins or some combination of both.

There are at least three possible explanations, none mutually exclusive, for the patterning of within-lake genetic divergence reported amongst the Lomond *C. lavaretus* here. Probably the most parsimonious is that this weak structuring could represent the very earliest stages of population fragmentation leading to the within-lake divergence reported elsewhere in this species (Præbel *et al.,* 2013a; Siwertsson *et al.,* 2013) and related species (Gislason *et al.,* 1999; Adams *et al.,* 2008; Garduño-Paz *et al.,* 2012). There is some additional supporting evidence that the genetic structuring described here is also underpinned by ecological differences between fish from different basins. Etheridge and her colleagues (Etheridge *et al.,* 2010) showed structuring in foraging ecology in *C. lavaretus* across basins in Loch Lomond. Stable isotopes ratios of C and N from muscle tissue of *C. lavaretus* in winter, indicative of the foraging pattern over the previous summer, differed between fish from the north, south and mid basins of Lomond. The population fragmentation described for Loch Lomond here appears to be based on broad scale lake characteristics (basins), this differs from the intra-lacustrine patterns described for the same species in northern Norway and Finland, where although there is some spatial patterning of phenotypically and genetically distinct groups, this is based upon habitat use differences with water depth (Siwertsson *et al.,* 2012).

Although emerging population divergence is one (and probably the most likely) explanation for the patterns presented here, there are at least two others. There is the possibility that the subtle genetic patterning is the result of previously divergent

populations which have subsequently collapsed. Merging of well-defined genetically and phenotypically diverged populations has been recorded at least twice previously for this and other freshwater fishes (Taylor *et al.,* 2006; Bittner *et al.,* 2010; Bhat *et al.,* 2014). However, there is no historical evidence for clearly defined, separate populations of European whitefish defined genetically or by phenotype in Loch Lomond, although equally there is also no robust evidence that such populations have not existed previously.

A third possibility is that the contemporary patterns amongst Lomond *C. lavaretus* are on neither a divergent nor a collapsing evolutionary trajectory, rather that the current structure represents some stable state driven by weak balancing selection forces resulting from spatial differences in selection caused by significant habitat heterogeneity. One plausible explanation is that some structuring that is driven by high levels of fidelity to spawning sites is prevented from becoming more extreme because of constant and repeat straying between geographically distinct spawning sites. Future analysis may benefit from including genetic tools offering higher resolution to fully understand the mechanisms that lie beneath this structuring.

The estimates of effective population size for the Loch Lomond and Loch Eck *C. lavaretus* revealed population sizes around the lower limit of the theoretical recommendation (Ne = 50) for a population that might be considered vulnerable to immediate effects of inbreeding and an order of magnitude smaller than recommended for maintaining genetic variation indefinitely (Allendorf & Ryman, 2002; Van Dyke, 2003). This strongly suggests that the adaptive potential to adjust to significant environmental perturbations in these populations may be limited. In conclusion, there is clear genetic and phenotypic structuring across native populations of *C. lavaretus* in Scotland that probably have originated from a common ancestor. Secondly, there was also detectable but weak genetic structuring between “sub-populations” within the large Loch Lomond. Thus the hypothesis that between lake structuring would be greater than within lake structuring and that the habitat heterogeneity of Loch Lomond would support genetic

structuring are both supported. A further test of the nature of the sub-structuring within Loch Lomond might focus on analysing larger sample sizes in combination with genetic methods of higher resolution, such as next generation sequencing. This pattern of within lake structuring may indicate the beginnings of a sympatric divergence of gene pools or a collapse of previously divergent groups. Alternatively weak balancing section with some spatial variation in selection may have resulted in these patterns. Whichever of these is in operation, these unique populations are of high conservation importance and their genetic structure suggests that they are vulnerable to future environmental perturbations.

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Tables

Table I. Sampling locations for *Coregonus lavaretus* in this study.

Site	Latitude (N)	Longitude (W)
1. Lomond - North	56° 16′ 58.4″	04° 41′ 22.2″
2. Lomond - North	56° 16′ 42.7″	04° 41′ 47.5″
3. Lomond - North	56° 16′ 37.5″	04° 41′ 49.6″
4. Lomond - North	56° 16′ 14.3″	04° 40′ 36.6″
5. Lomond - North	56° 16′ 37.5″	04° 41′ 49.6″
6. Lomond - Mid	56° 7′ 33.3″	04° 36′ 48.9″
7. Lomond - Mid	56° 7′ 25.9″	04° 37′ 46.4″
8. Lomond - Mid	56° 6′ 36.2″	04° 36′ 23.1″
9. Lomond - South	56° 2′ 10.0″	04° 37′ 35.6″
10. Lomond - South	56° 1′ 54.6″	04° 37′ 34.3″
1. Eck - North	56° 6′ 21.1″	04° 59′ 26.6″
2. Eck - South	56° 2′ 47.1″	04° 59′ 04.8″

Table II. Summary of the genetics samples included in the study. Lakes sampled, Location of basin, sample abbreviations (Code), sample size (N), expected heterozygosity (H_e), the inbreeding coefficient F_{is}, and the significance from the HWE test (P_{HWE}), mean allelic (N_{AR}) and mean private allelic richness (N_{PAR}) determined by the rarefaction procedure as implemented in HP-RARE (Kalinowski 2005), and the effective population size (N_e) with the lower and upper credible limits.

Lake	Location	Code	N	H_e	F_{is}	P_{HWE}	N_{AR}	N_{PAR}	N_e
Lomond	North	LLN	18	0.462	0.059	0.712	4.1±0.7	0.4±0.3	-
	Mid	LLM	20	0.450	-0.071	0.911	3.6±0.6	0.3±0.1	-
	South	LLS	21	0.491	0.027	0.614	3.9±0.5	0.4±0.2	-
Lomond	Pooled N+M+S		59	0.471	0.011	<0.001	-	-	-
Lomond	Pooled M+S	LLO	41	0.480	-0.028	0.371	4.7±0.6	1.2±0.3	54 (45-72)
Eck		ECK	44	0.422	0.016	0.969	3.8±0.5	0.4±0.1	52 (43-70)

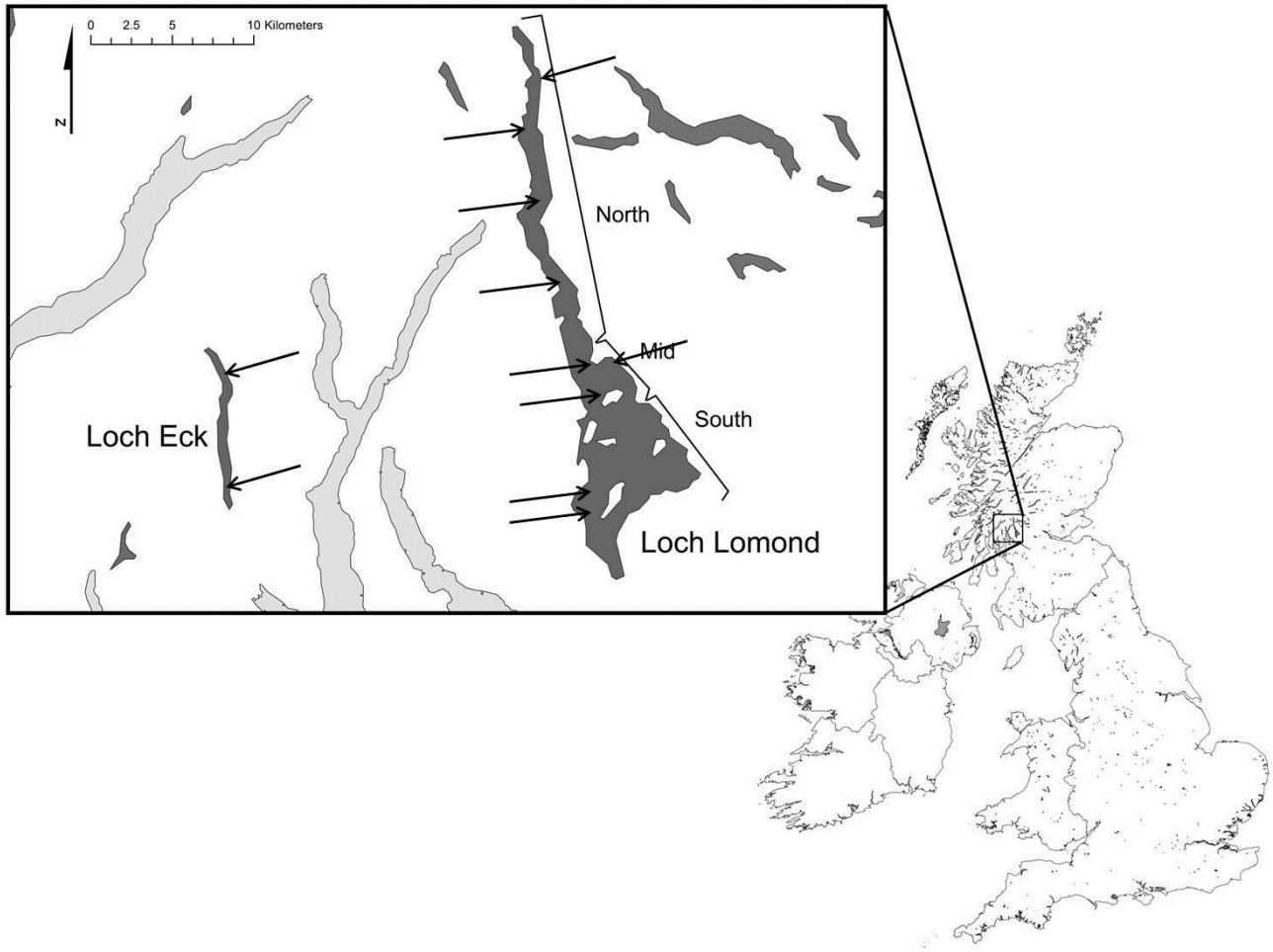
Legends

Figure 1. Loch Lomond and Loch Eck, Scotland showing the lake basin boundaries and the sampling sites (arrows) for *C. lavaretus*.

Figure 2 The location of the landmarks used to estimate shape of *C. lavaretus*. These are: 1 the most anterior point of the snout; 2 & 4 - the dorsal and ventral point of the eye socket (left side); 3 & 5 the anterior and posterior points of the eye socket (left side); 6 & 7 the

anterior and posterior points of the eye socket (right side); 9 the anus; 10 & 11 the lower and upper part of the caudal peduncle where is joins the caudal fin.

Figure 3. The Discriminant Function Analysis score frequencies and the shape change associated with the DFA score differences for *C. lavaretus* at first feeding from a common-garden experiment originating from fertilised eggs collected from Loch Eck (dark grey) and Loch Lomond (light grey). (Between location: Procrustees distance = 0.010; $P<0.001$; Mahalanobis distance = 1.81 $P<0.002$).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North
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Loch Eck
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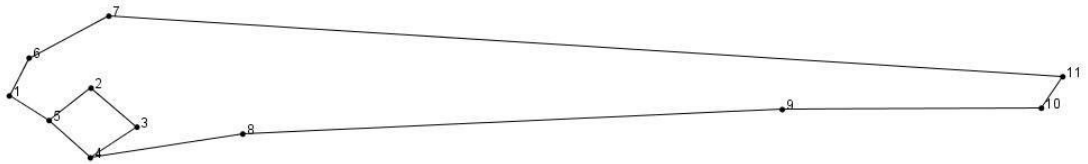
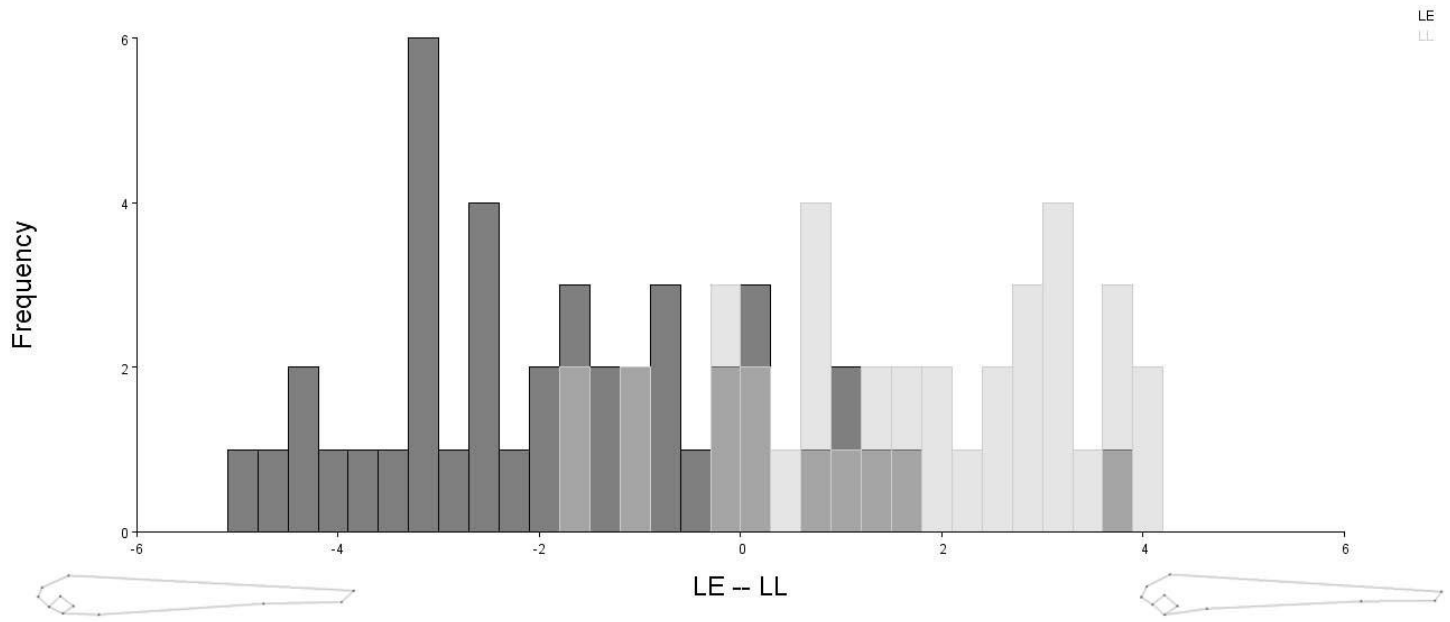


LE -- LL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Frequency
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Supporting Information

Table S1. Summary of sample sizes (N), number of alleles (N_A), observed (H_o) and expected (H_e) heterozygosity, and F_{is}-estimates of the studied populations and loci. Table-wide significance levels for H.-W. tests were applied for each locus separately (**bold** values). None of the p-values were significant after sequential Bonferroni correction (at the 5% level or less) following (Rice *et al.* 2008). BFRO-018 (Susnik *et al.* 1999), BWF1, BWF2 (Patton *et al.* 1997), C2-157 (Turgeon, Estoup & Bernatchez 1999), Cla-Tet01, Cla-Tet03, Cla-Tet10, Cla-Tet13, Cla-Tet15, Cla-Tet18 (Winkler & Weiss 2008b), Cocl-lav04, Cocl-lav06, Cocl-lav10, Cocl-lav18, and Cocl-lav49 (Rogers *et al.* 2004).

Pop		BWF1	Cla-Tet03	Cla-Tet13	Cla-Tet18	Cocl-lav06	Cla-Tet10	Cocl-lav18	Cocl-lav49	Cla-Tet15	C2-157	Cla-Tet01
LLN	**N**	18	18	18	18	18	18	18	18	18	18	18
	N_A	3	7	8	4	2	8	2	2	4	2	3
	H_o	0.167	0.667	0.833	0.278	0.389	0.778	0.278	0.167	0.556	0.389	0.278
	H_e	0.156	0.622	0.772	0.534	0.375	0.793	0.375	0.153	0.477	0.424	0.248
	F_{is}	-0.069	-0.072	-0.080	**0.480**	-0.037	0.019	0.259	-0.091	-0.165	0.084	-0.118
LLM	**N**	20	20	20	20	20	19	20	19	20	20	20
	N_A	2	5	5	3	2	8	2	2	5	3	3

	H_o	0.050	0.600	0.700	0.300	0.500	0.947	0.650	0.263	0.700	0.300	0.300
	H_e	0.049	0.503	0.721	0.261	0.480	0.788	0.499	0.361	0.590	0.329	0.265
	F_{is}	-0.026	-0.194	0.029	-0.148	-0.042	-0.202	-0.303	0.272	-0.186	0.087	-0.132
LLS	**N**	23	22	23	23	23	23	23	22	23	23	23
	N_A	5	7	4	5	2	6	2	2	5	2	5
	H_o	0.217	0.500	0.739	0.261	0.478	0.739	0.348	0.409	0.696	0.435	0.435
	H_e	0.202	0.448	0.680	0.458	0.466	0.752	0.386	0.375	0.652	0.454	0.403
	F_{is}	-0.075	-0.115	-0.088	**0.431**	-0.026	0.018	0.098	-0.091	-0.067	0.042	-0.080
LLO	**N**	40	40	41	41	41	40	41		41	41	41
	N_A	4	7	5	5	2	8	2		6	3	5
	H_o	0.125	0.550	0.732	0.268	0.463	0.850	0.512		0.707	0.366	0.366
	H_e	0.120	0.478	0.705	0.333	0.470	0.783	0.470		0.637	0.410	0.339
	F_{is}	-0.044	-0.150	-0.038	**0.194**	0.015	-0.086	-0.089		-0.110	0.108	-0.080
ECK	**N**	42	44	44	44	44	44	44		44	44	44
	N_A	3	6	5	4	2	7	3		3	2	4
	H_o	0.286	0.409	0.477	0.364	0.114	0.614	0.523		0.545	0.432	0.386
	H_e	0.281	0.403	0.481	0.382	0.107	0.673	0.464		0.551	0.425	0.399
	F_{is}	-0.015	-0.015	0.007	0.048	-0.060	0.088	-0.126		0.011	-0.015	0.030

Table S2. Pairwise F_{ST} estimates for *C. lavaretus* collected from each of the 3 basins in Loch Lomond for each locus individually. * P < 0.1

Locus: BWF1		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0192	
Lomond-M	0.0062	0.0142

Locus: ClaTet03		
Population	Lomond-N	Lomond-S
Lomond-S	0.0141	
Lomond-M	-0.0013	-0.0076

Locus: ClaTet15		
Population	Lomond-N	Lomond-S
Lomond-S	0.0240*	
Lomond-M	-0.0023	-0.0030

Locus: Cocl_lav18		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0301	
Lomond-M	0.0801*	0.0751

Locus: C2-157		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0234	
Lomond-M	0.0080	0.0377

Locus: ClaTet10		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0199	
Lomond-M	0.0172	0.0190*

Locus: ClaTet18		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0175	
Lomond-M	0.0654*	0.0049

Locus: Cocl_lav49		
Population	Lomond-N	Lomond-S
Lomond-S	0.0698	
Lomond-M	0.0525	-0.0272

Locus: ClaTet01		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0039	
Lomond-M	-0.0180	0.0000

Locus: ClaTet13		
Population	Lomond-N	Lomond-S
Lomond-S	-0.0108	
Lomond-M	-0.0073	-0.0071

Locus: Cocl_lav06		
Population	Lomond-N	Lomond-S
Lomond-S	0.0081	
Lomond-M	0.0243	-0.0217