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Introgression from extinct species facilitates adaptation to its vacated niche

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Abstract

Anthropogenic disturbances of ecosystems are causing a loss of biodiversity at an unprecedented rate. Species extinctions often leave ecological niches underutilized, and their colonization by other species may require new adaptation. In Lake Constance, on the borders of Germany, Austria and Switzerland, an endemic profundal whitefish species went extinct during a period of anthropogenic eutrophication. In the process of extinction, the deep-water species hybridized with three surviving whitefish species of Lake Constance, resulting in introgression of genetic variation that is potentially adaptive in deep-water habitats. Here, we sampled a water depth gradient across a known spawning ground of one of these surviving species, *Coregonus macrophthalmus*, and caught spawning individuals at greater depths (down to 90 m) than historically recorded. We sequenced a total of 96 whole genomes, 11–17 for each of six different spawning depth populations (4, 12, 20, 40, 60 and 90 m), to document genomic intraspecific differentiation along a water depth gradient. We identified 52 genomic regions that are potentially under divergent selection between the deepest (90 m) and all shallower (4–60 m) spawning habitats. At 12 (23.1%) of these 52 loci, the allele frequency pattern across historical and contemporary populations suggests that introgression from the extinct species potentially facilitates ongoing adaptation to deep water. Our results are consistent with the syngameon hypothesis, proposing that hybridization between members of an adaptive radiation can promote further niche expansion and diversification. Furthermore, our findings demonstrate that introgression from extinct into extant species can be a source of evolvability, enabling rapid adaptation to environmental change, and may contribute to the ecological recovery of ecosystem functions after extinctions.

KEYWORDS
adaptive radiation, deep-water adaptation, environmental change, extinction by hybridization, introgression

1 | INTRODUCTION

The recovery of ecosystems from anthropogenic disturbances is a central factor to predict future consequences of environmental change on biodiversity (Malhi et al., 2020). When species go extinct due to anthropogenic disturbance, previously occupied niche space may become vacant (Prada et al., 2016). Thus, in communities lacking ecological redundancy, extinction can provide surviving species with previously unavailable ecological opportunity (Prada et al., 2016; Wellborn & Langerhans, 2015). Whether and within which timescale such vacant niche space that had previously been occupied by newly extinct species can be filled again through niche expansion of related or newly emerging species is one critical aspect of determining the functional recovery of an ecosystem on an evolutionary timescale.

Degradation of ecosystems can result in species loss by either demographic decline, or by speciation reversal through the merging of several related species into a single hybrid population (Rhymer & Simberloff, 1996; Taylor et al., 2006; Todesco et al., 2016). When reproductive isolation between species is mediated by features of the environment that interact with intrinsic lineage traits, environmental change can induce the loss of reproductive isolation, resulting in speciation reversal through introgressive hybridization (Seehausen, 2006; Seehausen et al., 2008; Taylor et al., 2006, Todesco et al., 2016). Even when ecosystems are restored and thus disturbance was merely transient, speciation reversal can result in the loss of species. However, parts of the genetic variation that had once defined the lost species will then often have been transferred to surviving species through hybridization and introgression (Barlow et al., 2018; Kuhlwilm et al., 2019; Reilly et al., 2022). Thereby, speciation reversal can result in the functional extinction of taxa within a few generations (Rhymer & Simberloff, 1996; Taylor et al., 2006; Todesco et al., 2016; Vonlanthen et al., 2012), while some of their genetic variation may persist within extant species (Frei et al., 2022; Gilman & Behm, 2011).

Rapid adaptation and speciation are often associated with re-assembling of old genetic variation originating from hybridization (Hamid et al., 2021; Marques et al., 2019; Moran et al., 2021). Examples include Darwin's finches (Lamichhaney et al., 2015, 2016), cichlid fish (Irisarri et al., 2018; Meier et al., 2017), *Lycaeides* butterflies (Nice et al., 2013) and *Helianthus* sunflowers (Rieseberg et al., 2003). Hybridization is thought to promote ecological diversification and speciation because it can generate new trait combinations suitable for utilizing resources that could not be utilized before (Marques et al., 2019; Seehausen, 2004). Through these effects, hybridization can fuel entire adaptive radiations, both the onset (Meier et al., 2017) and the continuation of adaptive radiation beyond the first speciation events (Seehausen, 2004). In the context of extinction by speciation reversal, introgressive hybridization during speciation reversal might facilitate the adaptation of a surviving species to an extinct species' vacated niche. Admixture variation generated through hybridization during speciation reversal might be re-assembled into allelic combinations that are adaptive in the now unoccupied habitat previously used by the extinct species. When some of the alleles derived from the extinct species that evolved in response to selective pressures in its former habitat introgress into surviving species, they might facilitate adaptation to the now vacant habitat within surviving species. Such a scenario is in line with the syngameon hypothesis of adaptive radiations, which predicts that hybridization between members of an existing adaptive radiation might induce further adaptation and diversification (Seehausen, 2004).

The Alpine whitefish radiation provides an outstanding opportunity to study the consequences of speciation reversal induced by severe but transient environmental change. Reproductive isolation between sympatric Alpine whitefish species is maintained predominantly by extrinsic (and possibly intrinsic) prezygotic and extrinsic postzygotic mechanisms (Hudson et al., 2016; Ingram et al., 2012; Vonlanthen et al., 2012; Woods et al., 2009). Many sympatric species differ in the water depth of spawning sites and show differential timing of spawning (Steinmann, 1950). Some sympatric species overlap in both, yet retain significant reproductive isolation, possibly due to behavioural mating preferences (Hudson et al., 2016; Steinmann, 1950). Alpine whitefish species are highly sensitive to alterations of the physiochemical habitat characteristics, because spawning niche differentiation and in turn reproductive isolation is strongly dependent on the persistence of fine-scale depth-related differences in the specific lacustrine habitat (Hudson et al., 2016; Vonlanthen et al., 2012). Anthropogenic eutrophication during the last century weakened reproductive isolation between Alpine whitefish species, resulting in speciation reversal through introgressive hybridization (Frei et al., 2022; Vonlanthen et al., 2012).

In Lake Constance, a lake between the borders of Germany, Austria and Switzerland, four endemic whitefish species have been taxonomically described. The deep-water species *Coregonus gutturosus* went extinct during eutrophication-induced speciation reversal (Vonlanthen et al., 2012). During the period of anthropogenic eutrophication, its deep-water spawning grounds were lost as a result of decreased oxygen concentrations (Nümann, 1972; Wahl & Loffler, 2009). The anoxic conditions at the water–sediment interface in deep benthic areas of the lake probably prevented successful reproduction of *C. gutturosus* and thereby contributed to its extinction (Deufel et al., 1986; Wahl & Loffler, 2009). Recent work demonstrated extensive introgression of this extinct species into all surviving members of the radiation (Frei et al., 2022). Introgression included potentially adaptive alleles that, before eutrophication, had been under positive selection in the extinct species (Frei et al., 2022). Today, oligotrophic conditions of the lake have been largely restored and deep-water habitats are again accessible for fish. Yet, profundal habitats of Lake Constance are reported to be devoid of any whitefish (Alexander & Seehausen, 2021). However, the genetic variation that had evolved in the extinct deep-water species and introgressed into the extant species during eutrophication-induced speciation reversal may provide the surviving species with alleles that could be adaptive in deep water (respectively adaptive in a now vacant profundal habitat previously occupied by the recently extinct species). Thus, introgression from the extinct profundal *C. gutturosus* could in principle facilitate adaptation to deep water habitats in some of the surviving Lake Constance whitefish species that had not occupied these greater depths previously.

Here, we sampled a depth transect on known spawning grounds of *Coregonus macrophthalmus*, the deepest spawning of the extant species. Adaptation to the extinct species' former deep-water habitat seems most plausible in this species. We set nets in six depth zones ranging from 4 to 120 m water depth during the spawning season. Thus, we sampled the entire historically known Lake Constance whitefish depth range from that of the shallowest spawning *C. macrophthalmus* populations down to the depths (90–120 m) where the extinct *C. gutturosus* used to spawn. We then sequenced whole genomes of 11–17 individuals per depth (total $n = 96$) to search for signatures of differentiation and adaptation along the water depth

gradient. We demonstrate that the deepest caught *C. macrophthalmus* individuals from 90m depth show morphological and genetic differentiation from the shallower caught individuals, and we identify 52 candidate loci that might be under positive selection in deep water. At 12 (23.1%) of these loci, the allele frequency pattern across our six different spawning depth populations of *C. macrophthalmus* together with the allele frequencies in the historical populations of all Lake Constance whitefish species sampled before speciation reversal (from Frei et al., 2022) suggest that these alleles might have introgressed from the extinct *C. gutturosus* during the period of anthropogenic lake eutrophication. Thus, our results demonstrate that some alleles that are likely to have introgressed from the extinct species are potentially involved in adaptation of some populations of *C. macrophthalmus* to the deep-water environment historically used as habitat by the now extinct *C. gutturosus*. This suggests that introgressive hybridization during speciation reversal potentially facilitates adaptation within surviving species to the vacated habitat of a recently extinct species.

2 | MATERIALS AND METHODS

2.1 | Study system

In Lake Constance, a large pre-alpine lake bordering Germany, Austria and Switzerland, four whitefish species have been taxonomically described (Steinmann, 1950). *Coregonus wartmanni* is most relevant for commercial fisheries and is extensively managed. It is a pelagic species, mostly feeding on planktonic food resources in the open water (Steinmann, 1950). *C. wartmanni* spawns pelagically, close to the surface over deep water (70–250m) from late November until early December (Nenning, 1834; Nüsslin, 1907; Schweizer, 1894, 1926; von Rapp, 1858). *Coregonus macrophthalmus* is the second most imprortant for commercial fisheries. *C. macrophthalmus* feeds on both pelagic and benthic food resources (Steinmann, 1950). The species has historically been described to spawn on relatively shallow benthic spawning grounds at depths of less than 20m, close to the shore of the lake, starting from mid-November until early January (Nenning, 1834; Nüsslin, 1907; Eckmann & Rösch, 1998; Schweizer, 1894; Schweizer, 1926; Steinmann, 1950). More recent work has suggested an extended range of spawning depth of *C. macrophthalmus* between 2 and 50m after the lake had returned to an oligotrophic state (Hirsch et al., 2013; Jacobs et al., 2019). *Coregonus arenicolus* is a relatively large-bodied species, feeding on large benthic macroinvertebrates (Steinmann, 1950). It has a very short spawning period in mid-November and it has been historically described to spawn on very shallow spawning grounds of 1–2 m depth and mainly on sandy substrate (Nenning, 1834; Schweizer, 1894; Steinmann, 1950; von Rapp, 1858). *Coregonus gutturosus* went extinct during the period of anthropogenic eutrophication during the 1970s or 1980s (Vonlanthen et al., 2012). *C. gutturosus* was a deep-water specialist, feeding on benthic macroinvertebrates in the profundal regions of the lake (Steinmann, 1950). It had an extended spawning period ranging from summer to winter (Steinmann, 1950), during which it spawned benthically in depths around 70–80m or more (Steinmann, 1950; von Rapp, 1858; von Siebold, 1858).

2.2 | Sampling

Nets were set at seven different water depths (4, 12, 20, 40, 60, 90 and 120m) on a known *C. macrophthalmus* spawning ground at the beginning of the spawning season of 2019 (November 26 to 29). We used benthic gillnets with varying mesh sizes, consisting of panels of 25-, 35- and 45-mm mesh size to cover the known range of body sizes of spawning whitefish. We caught fish down to 90m, but not at 120m, suggesting that we covered the whole range of depth that is currently used by whitefish for spawning. Individuals were anaesthetized and subsequently euthanized using appropriate concentrations of tricaine methane sulphonate solutions (MS-222) according to a permit issued by the canton of St. Gallen (SG31396). Fin-clips were taken and stored in 100% analytical ethanol until extraction of DNA. Individual specimens were weighed, total length was measured, a standardized picture was taken and a first species assignment was made on site. All the fish caught were fixed in 4% formalin solution for 1 month, and then transferred through a series of increasing ethanol concentrations (pure water, 30%, 50%) to the final concentration of 70% for long-term storage.

2.3 | Morphometric analysis

On all fish caught, we measured 23 linear morphometric traits using digital callipers according to Selz et al. (2020), except for taking the mean of three measurements per trait (instead of the mean of two measurements). The traits measured were BD (body depth), DHL (dorsal head length), PreD (predorsal length), PostD (postdorsal length), CD (caudal peduncle depth), CL (caudal peduncle length), SL (standard length), HL (head length), HD (head depth), HW (head width), PostO (postorbital length), SN (snout length), ED (eye diameter), EH (eye height), SD (snout depth), SW (snout width), M (length of maxilla), MW (mouth width), UJ (upper jaw length), LJ (lower jaw length), LJW (lower jaw width), IOW (interorbital width) and INW (internarial width) (see Table 1 in Selz et al., 2020). Additionally, we counted the number of gill-rakers (GRC) also according to Selz et al. (2020). We used individuals that were assigned to *C. macrophthalmus* ($n = 106$) for the following morphological analyses. First, we size-corrected our 23 linear morphometric traits by using the residuals of the linear regression of standard length with the specific trait for further analysis. To assess morphological differentiation between fish caught at different depths, we performed a partial least squares regression analysis between all size-corrected traits (excluding standard length and gill-raker count) and depth (4, 12, 20, 40, 60 and 90m) in R (R Core Team, 2018) using the package "PLS" (Mevik & Wehrens, 2007). Finally, we tested whether the first component

was significantly correlated with depth using a Spearman correlation in R (R Core Team, 2018) to see if morphological differentiation is associated with water depth.

2.4 | DNA extraction and sequencing

We sequenced all individuals caught in the 4-, 12-, 40-, 60- and 90-m net. Only for the 20-m net, where we caught a total of 31 individuals, we randomly downsampled the number of individuals to 16 to achieve a balanced sampling across all depths. DNA was extracted from fin clips with the Qiagen DNeasy blood and tissue kit (Qiagen), using the standard protocol for tissue samples supplied by the manufacturer. DNA concentrations were quantified on a Qubit 2 fluorometer (Thermo Fisher Scientific). An Illumina paired-end TruSeq DNA PCR-Free library (Illumina) was prepared for each fin-clip sample. Library preparation was performed by the next-generation sequencing (NGS) platform of the University of Bern following the manufacturer's instructions. Libraries were then paired-end sequenced at 150 bp on an Illumina Novaseq 6000 S4 flow cell. Individual sequencing coverage at polymorphic sites (see next section, called across all 91 *C. macrophthalmus* individuals sequenced and with data from at least 85 individuals at each position) was on average ~8.6× and ranged between ~4.3× and ~16.1× in the 91 sequenced *C. macrophthalmus* individuals. Individual coverage did not differ significantly between the different sampling depths according to a one-way ANOVA ($p = .163$) performed in R (R Core Team, 2018). Mean coverage was ~8× (range between ~5.6× and ~13.6×) in the 4-m spawning depth population, ~7.4× (range between ~4.3× and ~13×) in the 12-m spawning depth population, ~8.1× (range between ~4.7× and ~13.9×) in the 20-m spawning depth population, ~10.4× (range between ~6.2× and ~16.1×) in the 40-m spawning depth population, ~10× (range between ~5× and ~14.8×) in the 60-m spawning depth population, and ~8.1× (range between ~5.1× and ~14.1×) in the 90-m spawning depth population.

2.5 | Processing reads and mapping

Raw reads were processed and mapped to the Alpine whitefish reference genome following Frei et al. (2022). In brief, poly-G tails were removed using FASTP 0.20.0 (Chen et al., 2018) and overlapping read pairs with overlaps longer than 25 bp were subsequently merged using SEQPREP 1.0 (https://github.com/jstjohn/SeqPrep). The processed reads were then mapped to the Alpine whitefish reference genome (De-Kayne et al., 2020) using BWA 0.7.12 (Li & Durbin, 2009) adjusting the "r" parameter to 1. We marked duplicate reads, fixed mate information and replaced read groups (settings used except for the default parameters were VALIDATION_STRINGENCY = LENIENT and MAX_FILE_HANDLES_FOR_READ_ENDS_MAP = 1024) using PICARD TOOLS 2.20.2 (http://broadinstitute.github.io/picard/).

2.6 | Population genomic analysis

We then used ANGSD version 0.925 (Korneliussen et al., 2014) to calculate genotype likelihoods across all 96 samples caught at the six different depths, and additionally included all historical individuals of Frei et al. (2022) (short read archive accession PRJEB43605) to verify species assignment made in the field. Only sites covered with at least two reads in at least 118 individuals (out of a total of 128 individuals) and passing a *p*-value cut-off of 10 E-6 for being variable were included, while all sites with more than two alleles were excluded. Reads that did not map uniquely to the reference and had a mapping quality below 30, as well as bases with quality score below 20 were not considered. The following *p*-value cut-offs for single nucleotide polymorphism (SNP) filters implemented in ANGSD version 0.925 (Korneliussen et al., 2014) were used: -sb_pval 0.05 -qscore_pval 0.05 -edge_pval 0.05 -mapq_pval 0.05. To verify species assignment based on the resulting 941,976 SNPs with minor allele frequency above 0.05 (default parameter of PCANGSD 0.98), we performed a principal components analysis (PCA) and calculated admixture proportions based on the thirst three eigenvectors (−e 3) using PCANGSD 0.98 (Meisner & Albrechtsen, 2018). In total, we identified one individual belonging to *C. wartmanni* (or possibly being an early generation hybrid) and four individuals belonging to *C. arenicolus* (matching our species assignment made in the field) and thus these five samples were excluded from subsequent analysis. We used a generalized linear model (glm) in R (R Core Team, 2018) to test whether the *C. gutturosus* admixture proportions were different between the different depths that we sampled.

We then used 11 *C. gutturosus* individuals and the two historical *C. macrophthalmus* individuals from Frei et al. (2022) in combinations with the 91 *C. macrophthalmus* individuals caught at either 4, 12, 20, 40, 60 or 90 m to test for introgression from *C. gutturosus* into each spawning depth population separately, using the population-based D-statistics (Soraggi et al., 2018) implemented in ANGSD 0.925 (Korneliussen et al., 2014). We first calculated genotype likelihoods using only the 104 above-mentioned samples and using the same parameters as described above but adjusting the missing data parameter to include sites with data from at least 99 individuals. This resulted in a total of 517,250 SNPs that were then used for the D-statistics. We used a *Salmo salar* individual from Kjaerner-Semb et al. (2016) (short read archive accession: SSR3669756) as outgroup P4, the 11 *C. gutturosus* individuals as donor population P3, all *C. macrophthalmus* individuals of one of the six sampled depths as P2 and the two historical *C. macrophthalmus* as P1. Based on this ordering of populations on the four-taxon topology, it is possible to test for excess allele sharing between *C. gutturosus* and post-eutrophication populations of extant species relative to the same species sampled pre-eutrophication, which would be indicative of introgression of *C. gutturosus* into this species during eutrophication. We then repeated the analysis but replaced the donor population P3 with all historical *C. arenicolus* and *C. wartmanni* individuals from Frei et al. (2022) to test for introgression of *C. arenicolus* or *C. wartmanni* respectively into our six *C. macrophthalmus* spawning depth

populations that must have occurred during eutrophication-induced speciation reversal.

We then calculated genotype likelihoods again, using the same parameters as above but only using the 91 *C. macrophthalmus* samples and adjusting the missing data parameter to include sites where data for at least 85 individuals were available. We used the resulting genotype likelihoods at 1,948,989 polymorphic sites to calculate (weighted) F_{ST} (Bhatia et al., 2013) between all possible pairs of spawning depth populations (as well as between 90 m and all other spawning depth populations pooled) in ANGSD 0.925 (Korneliussen et al., 2014) based on one- and two-dimensional site frequency spectra inferred from site allele frequencies (Nielsen et al., 2012). We then again calculated a PCA with PCANGSD (0.98) (Meisner & Albrechtsen, 2018) at the 1,126,828 SNPs with minor allele frequency above 0.05 (default parameter of PCANGSD 0.98) to visualize population structure across depth within *C. macrophthalmus*. We further performed a selection scan along PC1 using PCANGSD (0.98) (Meisner & Albrechtsen, 2018) according to the method proposed by Galinsky et al. (2016). The method identifies unusual allele frequency shifts along previously inferred PC axes, making use of the fact that the squared correlation of each SNP to a specific PC axis, rescaled to account for genetic drift, follows a chi-square distribution (1 *df*) under the null hypothesis of the absence of selection (Galinsky et al., 2016). As PC1 separated the fish caught at 90 m from all the fish caught at shallower depths (4, 12, 20, 40, 60 m), this selection scan would detect positions that are under selection in deep water, respectively involved in depth adaptation. Following Pinsky et al. (2021), we FDR (false discovery rate)-corrected the resulting *p*-values and assumed SNPs with an FDR-corrected *p*-value <.05 to be under selection. The *p*-values were then log transformed for plotting using R (R Core Team, 2018).

At the 107 SNPs above the FDR-corrected significance threshold from the PCA-based selection scan, we used ANGSD 0.925 (Korneliussen et al., 2014) to calculate allele frequencies from genotype likelihoods of each spawning depth population separately using the method described in Kim et al. (2011), and we fixed the tracked allele to represent the reference allele of the Alpine whitefish reference genome. To remove redundant sites in strong physical linkage, we only considered positions that are more than 5 Mb apart from each other. We retained 52 SNPs for further analysis. We then additionally calculated the allele frequency in all historical *C. gutturosus* (n = 11) individuals, as well as in all historical *C. macrophthalmus* (n = 2), *C. arenicolus* (n = 3) and *C. wartmanni* (n = 2) individuals from Frei et al. (2022). SNPs that have a minor allele frequency above 0.05 in *C. gutturosus*, but are absent from all historical *C. macrophthalmus*, *C. arenicolus* and *C. wartmanni* have potentially been characteristic for *C. gutturosus* before the eutrophication period. Considering that our data showed that there was significant *C. gutturosus* introgression, detecting an allele with such a frequency pattern in contemporary populations of the extant species suggests that this allele introgressed from *C. gutturosus* during the anthropogenic eutrophication period. Following this logic, we looked for SNPs with such an allele frequency pattern consistent with *C. gutturosus* introgression

among the 52 independent SNPs inferred to be under selection between deep and shallower spawning *C. macrophthalmus* to find SNPs with alleles that potentially introgressed from *C. gutturosus* that may now facilitate deep-water adaptation in deep spawning *C. macrophthalmus*. We tested by permutation if the 52 sites potentially under selection between deep and shallower caught *C. macrophthalmus* are significantly enriched for SNPs with an allele frequency pattern consistent with *C. gutturosus* introgression. We randomly subsampled 52 positions (the same number as inferred to be under selection between deep and shallower spawning *C. macrophthalmus*), and then calculated the proportion of these subsampled SNPs that show an allele frequency pattern consistent with *C. gutturosus* introgression. We repeated this random subsampling 10,000 times to generate a null expectation, and then calculated a *p*-value by comparing the expected proportion of sites showing an allele frequency pattern of *C. gutturosus* introgression of these 10,000 permutations with the observed proportion calculated within the 52 sites potentially under selection between deep and shallower spawning *C. macrophthalmus*.

Finally, we assessed if the 107 SNPs (in 52 independent genomic regions) inferred to be under selection fall within genes, and if yes, in which genes. Gene annotations (from the Alpine whitefish genome [De-Kayne et al., 2020]; ENA accession: GCA_902810595.1) that overlap with the loci potentially under selection were identified using BEDTOOLS version 2.28.0 (Quinlan, 2014). We then used the protein sequence of the overlapping gene from the Alpine whitefish genome (De-Kayne et al., 2020; ENA accession: GCA_902810595.1) to perform a protein–protein BLAST (BLASTP) search against all genes of the annotation of the *S. salar* genome (taxid 8030). We reported the best hit for each gene (Table S4).

3 | RESULTS

3.1 | Sampling populations of *C. macrophthalmus* along a spawning depth gradient

We sampled the entire known spawning depth range of *Coregonus macrophthalmus* (4, 12, 20, 40 m), as well as greater depths where the extinct *Coregonus gutturosus* used to spawn (60, 90, 120 m) during the *C. macrophthalmus* spawning season from the end of November 2019 (see Figure 1a). Our sampling time point was in the middle of the typical spawning season of the targeted *C. macrophthalmus* (early November until early January), but also overlapped the spawning season reported for the now extinct *C. gutturosus*, ranging from July to early January (Steinmann, 1950). In total, we caught 106 *C. macrophthalmus* individuals, of which 93 (~88%) were fully ripe. While most fish were caught at 20 m (n = 31, Figure 1b), we caught spawning *C. macrophthalmus* individuals down to 90 m, but no fish were caught at the greatest depth fished (120 m). This suggests that our sampling covered the entire range of depth that is currently used for spawning by whitefish.

To verify our species assignment that was made in the field, we performed a genomic PCA (Figure S1) and a structure analysis
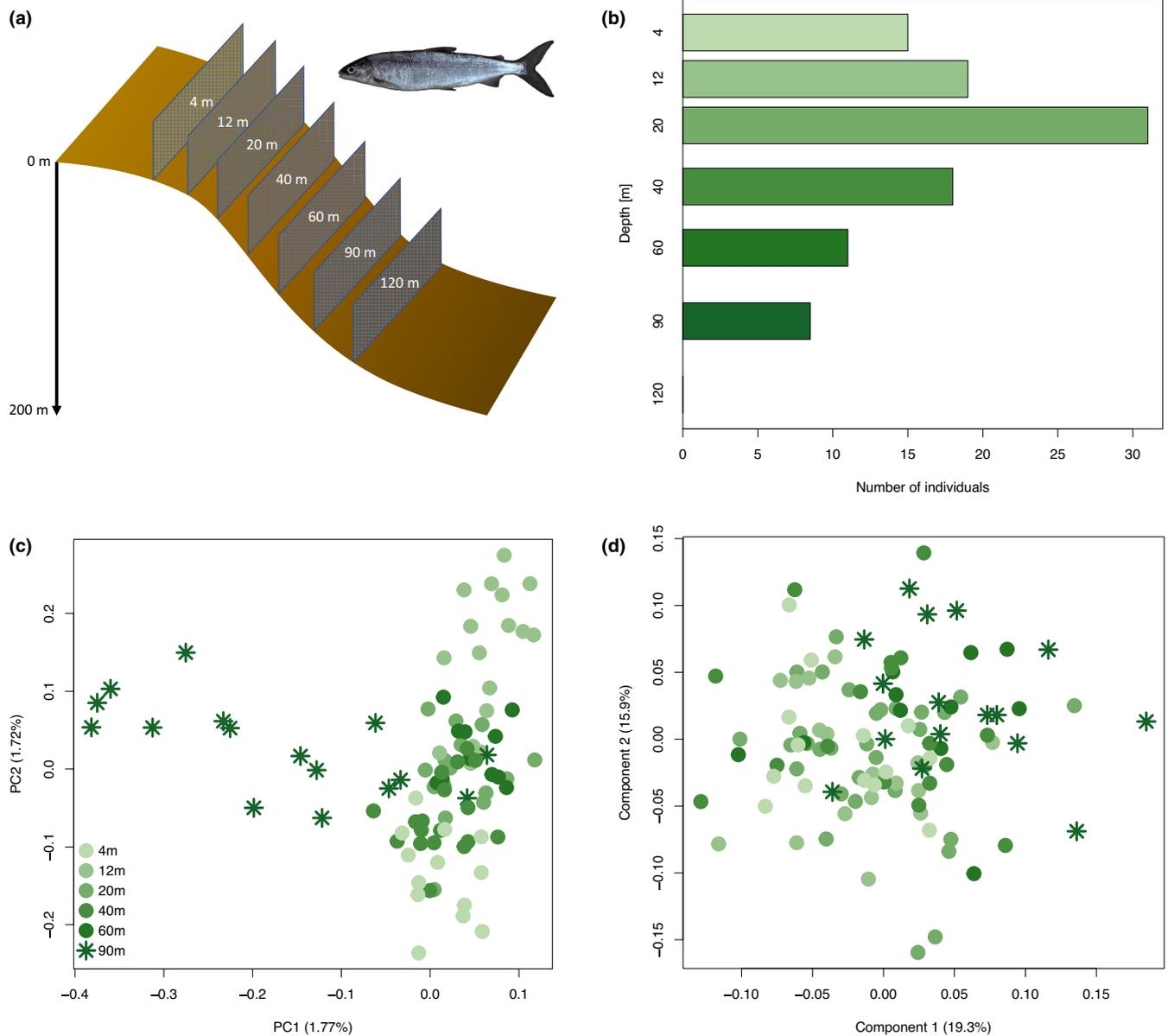
FIGURE 1 Differentiation along a water depth gradient. (a) Schematic overview of the sampling structure with nets set at seven different depths. (b) Number of individuals caught at each depth within 18 h (same sampling effort for each depth). (c) Genomic variation based on 1,126,828 polymorphic sites illustrated by a principal component analysis (PCA). The depth category of each individual is indicated by different green shadings (the deeper the darker). The 90-m spawning depth population is highlighted with asterisks, while all other spawning depth populations are indicated by dots. (d) Morphological differentiation is displayed as the two major components resulting from the partial least squares regression analysis. Symbols and colours are the same as in (c)

(Figure S2) using genotype likelihoods of 941,976 SNPs. We included all 96 sequenced individuals, as well as historical and contemporary individuals used in Frei et al. (2022) for reference. In both genomic PCA and structure analysis (Figures S1 and S2), four individuals of a total of 96 turned out to belong to *Coregonus arenicolus*, and thus were subsequently excluded from further analysis. One individual caught at 90 m clustered with *Coregonus wartmanni* in the PCA and resembled an early generation hybrid in the structure analysis. This fish was also excluded from all subsequent analyses to ensure that the results reflect solely the variation within *C. macrophthalmus* (n = 91).

3.2 | Morphological and genomic differentiation along the spawning depth gradient

We performed a partial least squares regression analysis based on linear morphometric measurements of 23 body and head traits (size corrected by using residuals of the linear regression against standard length) against spawning depth on all 106 individuals assigned to *C. macrophthalmus* (91 individuals randomly selected for sequencing, plus the 15 individuals that were caught but not sequenced). We found indications for morphological differentiation with depth (Figure 1d), with component 1 being significantly correlated with depth (rho = −0.44,

p = 2e-06, Figure S3). The traits with the highest loadings on both component 1 (LJW = 0.70, SD = 0.69 and MW = 0.39) and component 2 (SW = −0.55, EH = −0.49 and SNL = −0.35) were related to mouth (and head) shape (Figure S4). In contrast, our analysis of the genomic data did not yield any evidence for genomic differentiation along the spawning depth gradient when genome-wide F_{ST} was used as the test metric. We performed a genomic PCA based on genotype likelihoods of 1,126,828 SNPs in all sequenced individuals genetically assigned to *C. macrophthalmus* (n = 91; out of a total of 96 individuals that were randomly selected for sequencing). We found that the principal component explaining most variation (PC1, Figure 1c) separates the deepest caught individuals (90m) from all others, but the genome-wide F_{ST} between the 90-m sample and all shallower caught individuals did not differ from zero (weighted F_{ST} = −0.001567; all pairwise genome-wide F_{ST} values between depth categories were below zero). Similar to the morphological results, genomic PC1 was significantly correlated with depth (rho = −0.37, p =.0003, Figure S3). Taken together, our high-resolution data allowed us to identify subtle intraspecific differentiation within *C. macrophthalmus* in both genomic and morphological data (Figure 1c,d). The correlation of both morphological and genomic variation with depth suggests that the observed differentiation may be related to the onset of adaptation to deep water. Even though we did not observe genome-wide differentiation based on genome-wide F_{ST} estimates, which reflect neutral demographic processes that affect all SNPs (as the majority of SNPs along the genome are expected to evolve neutrally), our PCA approach demonstrates intraspecific differentiation within *C. macrophthalmus* between fish spawning at 90m and all shallower spawning individuals. However, this pattern might be driven by relatively few loci, which potentially show differentiation as a consequence of selective processes.

3.3 | Introgression from extinct deep-water species

We tested whether the six different *C. macrophthalmus* spawning depth populations (n = 11–16) received significant introgression from either *C. gutturosus*, *C. arenicolus* and/or *C wartmanni*, by making use of the historical samples of Frei et al. (2022). We detected significant introgression from *C. gutturosus* and *C. wartmanni* into each of our six *C. macrophthalmus* spawning depth populations (n = 11–16; Tables S1 and S2), but we did not detect significant introgression from *C. arenicolus* (Table S3). Per-individual *C. gutturosus* admixture proportions were not different between different depths (see Figure 2; p =.616 in a generalized linear model). However, the variance in admixture proportion was highest in the two deepest nets (60 and 90m) and the individual with the highest admixture proportion (~14%) was caught at 60m.

3.4 | Identifying genomic positions shaped by selection along the water depth gradient

We performed the selection scan proposed by Galinsky et al. (2016) implemented in PCANGSD (Meisner et al., 2021; Meisner & Albrechtsen, 2018) to find positions under selection between the *C. macrophthalmus* spawning depth populations. The method works best with data that are continuously distributed in PC space (Galinsky et al., 2016; Meisner & Albrechtsen, 2018) (see Figure 1c) and identifies positions that significantly deviate from genetic drift along an axis of differentiation (Meisner et al., 2021). As our PC1 (see Figure 1) separates most of the 90-m depth *C. macrophthalmus* from all samples from shallower depths, positions that are detected to be under selection along this PC axis would thus potentially be involved in adaptation to deep water. In total, we found 107 outlier SNPs (FDR-corrected p <.05) in 52 independent genomic regions (at least 5 Mb apart from each other) that are potentially under selection between the 90-m spawning site and shallower sites (Figure 3a). These 107 outlier SNPs overlapped a total of 30 genes (see Table S4).

3.5 | Allele frequency patterns consistent with introgression from extinct deep-water species

To assess whether adaptation to deep water in *C. macrophthalmus* was potentially facilitated by alleles introgressed from *C. gutturosus*,
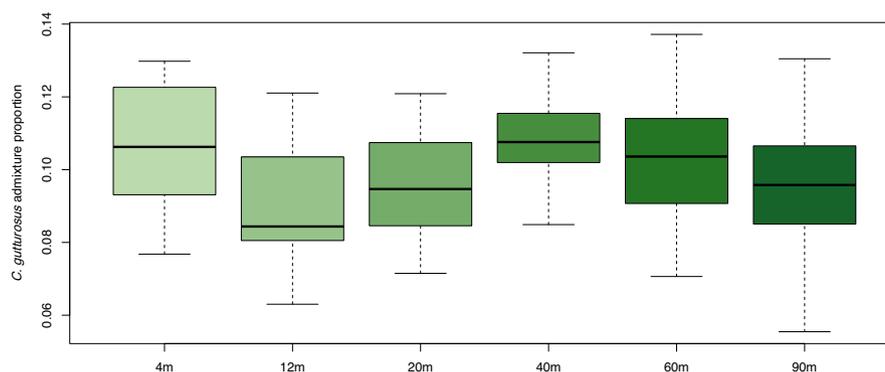

FIGURE 2 No differences in admixture proportions between spawning depth populations. Boxplots showing the *Coregonus gutturosus* admixture proportions from the PCANGSD admixture analysis (see Figure S2) in each spawning depth population. Horizontal bars correspond to medians, and whiskers to 1.5 times the interquartile range. There were no significant differences in admixture proportions between spawning depth populations (p = .616 in a generalized linear model)
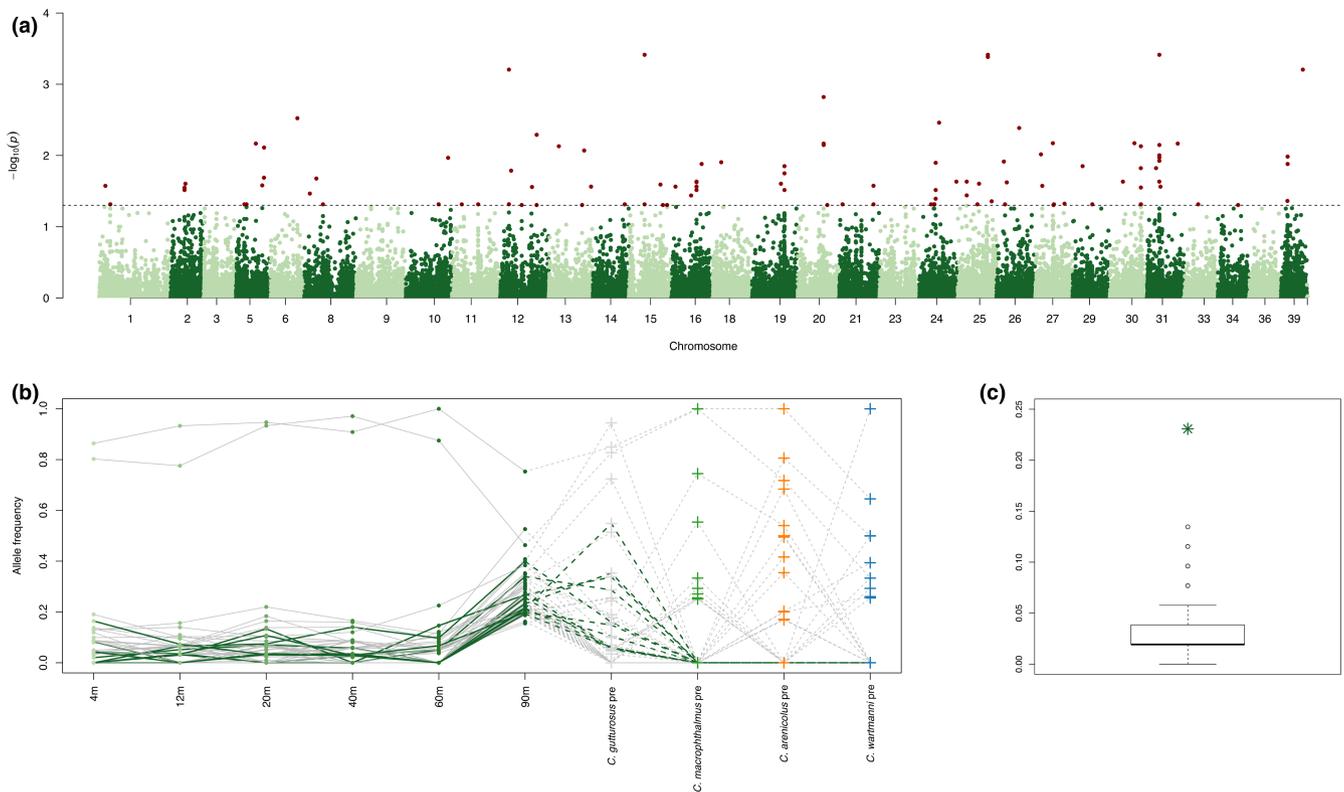
FIGURE 3 *Coregonus gutturosus* introgression is enriched at positions under selection between shallow and deep water. (a) Selection scan along PC1 from our genomic PCA (see Figure 1c) from Galinsky et al. (2016) as implemented in PCANGSD (Meisner et al., 2021; Meisner & Albrechtsen, 2018). As PC1 separates the 90-m population from shallower spawning *Coregonus macrophthalmus* populations, this approach identifies positions potentially under selection between deep and shallow spawning individuals. Shown are log-transformed and FDR-corrected *p*-values. The dashed line indicates the FDR-corrected .05 significance threshold, and all positions with *p*-values below the threshold are coloured in dark red. (b) Allele frequencies in the six different spawning depth populations (4–90 m) and in historical populations from Frei et al. (2022) (indicated with crosses and denoted with "pre"; grey for *C. gutturosus* [*n* = 11], green for *C. macrophthalmus* [*n* = 2], orange for *Coregonus arenicolus* [*n* = 3] and blue for *Coregonus wartmanni* [*n* = 2]). Shown are the 52 positions with an FDR-corrected *p*-value below.05 and at least 5 mb apart from each other. SNPs derived from *C. gutturosus* and potentially introgressed into *C. macrophthalmus* during eutrophication (frequency in *C. gutturosus* above 0.05, but allele is absent from all other historical populations) are coloured in green. (c) The distribution of 10,000 permutations of 52 randomly sampled positions (same number as shown in Figure 2b) along the genome, showing the proportion of alleles derived from *C. gutturosus* and potentially introgressed into *C. macrophthalmus* during eutrophication (same pattern as dark green trajectories in panel b). The green asterisk indicates the observed value (12 out of 52 loci in panel b)

we assessed the population allele frequency in our six spawning depth populations (4, 12, 20, 40, 60 and 90 m) at the 52 independent genomic positions with evidence for selection between the deepest (90 m) and all shallower (4–60 m) spawning *C. macrophthalmus* populations. Additionally, we also inferred allele frequencies at the same positions in 11 historical *C. gutturosus* individuals, two historical *C. macrophthalmus* individuals, three historical *C. arenicolus* individuals and two historical *C. wartmanni* individuals sampled from before the onset of eutrophication from Frei et al. (2022). At 12 out of 52 (23.1%) positions under selection between deep and shallower spawning *C. macrophthalmus*, we found that the alternative allele was present in *C. gutturosus* before the eutrophication period, while the allele was absent in the historical *C. macrophthalmus*, *C. arenicolus* and *C. wartmanni* samples from Frei et al. (2022) (Figure 3b). This pattern of allele frequencies across populations and species suggests that these alleles, potentially involved in adaptation to deep water in *C. macrophthalmus*, probably introgressed from *C. gutturosus* during the anthropogenic eutrophication period. Sites with such

an allele frequency pattern consistent with *C. gutturosus* introgression were significantly enriched among the 52 independent sites that are potentially under selection between deep and shallower caught *C. macrophthalmus* ($p < 10e-4$ obtained with 10,000 permutations, Figure 3c), suggesting that introgressed alleles from *C. gutturosus* may facilitate adaptation to deep water in *C. macrophthalmus*.

4 | DISCUSSION

Anthropogenic eutrophication of Lake Constance during the last century resulted in the extinction of the endemic profundal whitefish species *Coregonus gutturosus*, caused by a combination of demographic decline and speciation reversal through introgressive hybridization with other species of the same radiation. Introgression during speciation reversal resulted in the persistence of considerable parts of genomic variation from the extinct species within several extant species (Frei et al., 2022). We here show that one of the surviving Lake

Constance whitefish species, *Coregonus macrophthalmus*, is currently re-populating the deep-water environment that was left vacated after the extinction of *C. gutturosus*. Our systematic sampling of a spawning depth gradient demonstrated that today, *C. macrophthalmus* is spawning in greater depths (down to 90m) than previously reported for this species—less than ~20m before eutrophication in, for example, Nüsslin ([1907](#)) and Schweizer ([1926](#)), or ~20m in Eckmann and Rösch ([1998](#)) and 2–50m in Jacobs et al. ([2019](#)) after eutrophication. Our data suggest that introgression from *C. gutturosus* that occurred during its decline is potentially facilitating ongoing adaptation to the vacated deep-water niche in *C. macrophthalmus*.

4.1 | Re-population of and adaptation to the vacated deep-water environment

Adaptation to deep-water conditions at the lower end of a species' depth range is expected to result in morphologically and genomically localized rather than genome-wide differentiation between populations spawning deep and those that spawn at shallower depth, as selection is thought to favour phenotypes or combinations of alleles that increase fitness in deep-water habitats. Founder effects during range expansion might mimic adaptation, and hence could be mistaken for signals of adaptation. However, a founder effect would require some degree of geographical isolation between founder and source populations. As *C. macrophthalmus* has expanded its ecological niche at a spatial scale that lies within the dispersal distance of a single individual, a founder effect in the 90-m spawning depth population is unlikely. Furthermore, a founder effect in the deepest spawning population should be associated with an increase of genetic drift due to reduced effective population size. As a consequence, genetic diversity would decrease, resulting in genome-wide differentiation to all other spawning depth populations. In contrast with this prediction, we did not find evidence for genomic differentiation measured with genome-wide F_{ST}, which would reflect genome-wide differentiation resulting from demographic processes. Our data provide evidence for both subtle morphological and genomic differentiation between the deepest-spawning (90m) and shallower (4–60m) spawning populations of *C. macrophthalmus*. This subtle genomic differentiation might be genomically localized rather than genome-wide, given the evidence for differentiation between the 90-m spawning depth population in the PCA (see [Figure 1c](#)), but no evidence for such differentiation when using genome-wide F_{ST}. Additionally, the major axis of genomic differentiation and of morphological differentiation were both correlated with spawning depth. This suggests that the observed intraspecific differentiation between the 90-m spawning depth population and all shallower spawning depth populations might indeed be a result of adaptation to the vacant deep-water niche.

Until recently, whitefish have been reported to be absent from deep-water habitats of Lake Constance (Alexander & Seehausen, [2021](#)). Thus, the colonization of and adaptation to deep water in *C. macrophthalmus* described here probably started only

recently. This is consistent with theoretical work that demonstrated that, when a species goes extinct through hybridization, the re-population of its habitat is likely when disturbance that led to reversal is of only short duration (Gilman & Behm, [2011](#)). Hybridization during the extinction process facilitates the re-emergence of a similar phenotype to that of the extinct species through a combination of alleles derived from surviving and the extinct species, finally enabling the re-population of the vacated habitat. Adaptation and diversification by re-assembling alleles from two hybridizing species into new adaptive trait combinations is thought to be orders of magnitudes faster than adaptation and speciation based on de novo mutation alone (Marques et al., [2019](#)), and thus might be an important process in rapid adaptation to changing environments.

4.2 | Introgression facilitates adaption to extinct species' habitat

Introgression from *C. gutturosus* during eutrophication-induced speciation reversal might have provided the contemporary *C. macrophthalmus* population with alleles that are adaptive in deep water. The significant introgression from *C. gutturosus* into *C. macrophthalmus* caught in any depth zone demonstrated by D-statistics in combination with the matching allele frequencies between *C. gutturosus* and our 90-m *C. macrophthalmus* sample suggests that parts of the adaptation to deep water in *C. macrophthalmus* could be based on selection on introgressed variation derived from *C. gutturosus*. Ecological selection on introgressed variation is predicted to result in biased ancestry around functionally relevant genomic regions (Moran et al., [2021](#)). However, as both positive and negative selection may act on genomic variation derived from introgression (as, for example, demonstrated for Neanderthal introgression into some populations of *Homo sapiens*)—see, for example, Huerta-Sanchez et al. ([2014](#)), Racimo et al. ([2015](#)), Reilly et al. ([2022](#)) and Harris and Nielsen ([2016](#))—determining the exact selective forces acting on a specific allele is complex and challenging (Moran et al., [2021](#)). At 12 out of 52 (23.1%) independent positions indicating signatures of positive selection (and thus potentially involved in adaptation to depth), the patterns of allele frequencies suggested that the allele with increased frequency in the 90-m spawning depth population might have introgressed from *C. gutturosus*. Even though the sample sizes for the historical populations of the three extant species are limited, our permutation approach demonstrates that alleles that probably introgressed from *C. gutturosus* are enriched at positions under divergent selection between the deepest (90m) and shallower spawning (4–60m) *C. macrophthalmus* populations. This suggests that introgression from the extinct *C. gutturosus* might facilitate adaptation to its former deep-water habitat in the extant *C. macrophthalmus*. These results are in line with recent work that demonstrated that adaptation based on variation derived from recent admixture events can be very rapid and take only a few generations (Hamid et al., [2021](#)). Further, our findings are consistent with

the syngameon hypothesis of adaptive radiations, predicting that hybridization between species within an adaptive radiation can promote further diversification and speciation (Seehausen, 2004). Especially when environments change, hybridization within an adaptive radiation might increase the genomic variation of individual species and thereby enhance their adaptive potential, enabling a faster evolutionary response to the novel selective pressures of a changing environment (Grant & Grant, 2019). Consequently, hybridization between members of an adaptive radiation might be especially relevant under environmental change, potentially facilitating the survival of several species or even all species in a radiation through elevated evolvability and faster adaptation to the changing environmental conditions.

4.3 | Ecological recovery through evolution

Anthropogenic environmental change is affecting ecosystems worldwide whilst a large portion of contemporary species diversity is sensitive to hybridization-driven dynamics (Grabenstein & Taylor, 2018). Thus, the potential for speciation reversal to affect evolutionary trajectories of species and lineages is enormous (Seehausen et al., 2008). Our results suggest that the colonization of a vacated niche could potentially occur on a short evolutionary timescale when adaptation of an extant species is facilitated by introgression of alleles from the species that occupied this niche in the past and now is extinct. Such introgressed alleles have already been tested by selection in the environment originally inhabited by the extinct species. These alleles probably have the potential to facilitate rapid adaptation of the recipient species to these environmental conditions, provided that the disturbance resulting in extinction through speciation reversal was transient and of short duration (Gilman & Behm, 2011). This highlights the importance of quick and efficient ecosystem restorations after anthropogenic disturbances to maximize the chance of ecological recovery through evolution.

Hybridization in response to homogenized environments can result in dramatic losses of biodiversity within a few generations (Grabenstein & Taylor, 2018; Taylor et al., 2006). However, hybridization can also facilitate adaptation when environments become more heterogeneous again and thus promote the evolution of new biodiversity (Moran et al., 2021). When alleles that have evolved in a now extinct species introgress into a surviving species, they can outlast the species they evolved in and potentially be re-used to adapt to the extinct species' vacated habitat, other habitats or changed environmental conditions. The role of hybrid populations has been controversial in conservation biology (Draper et al., 2021). However, hybrid populations with high genetic variation and in turn high evolvability, such as those resulting from speciation reversal, can be important for future evolutionary dynamics that could contribute to the ecological recovery of an ecosystem. In turn, efficient and informed conservation measures should consider the implications of the existence of such hybrid populations with high adaptive

potential and the evolutionary dynamics that can emerge from them, potentially contributing to the recovery of an ecosystem on a timescale that is much shorter than the usually assumed evolutionary timescales of millennia.

CONFLICT OF INTEREST

The authors declare no competing interests.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at 10.25678/0007FH.

DATA AVAILABILITY STATEMENT

The raw sequencing files are accessible on ENA SRA (PRJEB53050). Additional supporting data (including the morphological data, genotype likelihood files and ENA sample accessions of all historical samples used) are deposited on the eawag research data institutional collection (doi:10.25678/0007FH). Scripts used for data analysis are available on GitHub (https://github.com/freidavid/Lake_Constance_Depth_Transect).

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SUPPORTING INFORMATION

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