



UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics

## **The speciation landscape of European whitefish in Northern Fennoscandia**

The importance of deglaciation history, standing genetic variation and natural selection

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## Summary

During the last deglaciation, one clade of European whitefish (*Coregonus lavaretus* L.) colonized the lakes in the three contemporary watercourses of northern Fennoscandia (Pasvik, Tana and Alta) and diverged thereafter in sympatry. This thesis investigates the interplay between evolutionary processes and standing genetic variation in shaping phenotypic and genetic divergence, and how reproductive isolation is established and maintained in whitefish. In this thesis, I demonstrated how selection has shaped the whitefish population across a large spatial scale despite the clear effect of random genetic drift - presumably arising from the colonization history. The diversification of European whitefish follows a (non)parallel trajectory, as morphs in the different watercourses were characterised with various levels of phenotypic and genetic divergence, and signatures of both within and between lakes divergence were observed in all studies conducted within this thesis.

As the reproductive isolation between whitefish eco-morph is not complete, hybridisation between morphs occurs frequently, which may refer relatively weak postzygotic barriers to gene flow between morphs. When whitefish morphs adapt to a specific niche, they encounter different environment conditions, like water temperature and prey availability that are important factors determining size at sexual maturity, somatic growth, and gonad development. Consequently, allochrony together with spatial differences in spawning may reinforces the ecological divergence and help to build up barriers to gene flow between the whitefish morphs. Indeed, allochrony seems to be working towards stronger reproductive isolation, as the advancement of the maturity, as well as the proportion of mature individuals differed between morphs.

The results of this thesis are in line with the theory of ecological speciation and show how selection has shaped the European whitefish morphs towards stronger phenotype-environment correlation and more diversified populations, despite the decreasing amount of standing genetic variation between the three contemporary watercourses. Thus, the occurrence of whitefish morphs in the three watercourses may be an outcome of convergent evolution, where similar phenotypes have arisen from different genetic backgrounds.

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## List of papers and contributions (co-author statements)

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### Papers

The following papers are included in my PhD thesis:

I: Diversifying selection drives parallel evolution of gill raker number and body size along the speciation continuum of European whitefish

II: (Non)parallel patterns of standing genetic variation and phenotypic diversity in a speciation continuum of European whitefish in northern Fennoscandia

III: Allochrony as a potential driver for reproductive isolation in adaptive radiations of European whitefish ecomorphs

### Contributions (alphabetical order)

	<b>Paper I</b>	<b>Paper II</b>	<b>Paper III</b>
Concept and idea	KH, KP	KH	JBT, KP
Study design and methods	KH, KP, KØ	KH, KP, SB	JBT, KH, KP, SB
Data gathering and interpretation	KH, KKK, KP, P-AA	AS, KH, KKK, KP, KØ, P-AA, RK, SB	JBT, KH, KP, SB
Manuscript preparation	KH, KKK, KP, KØ, P-AA	AS, KH, KKK, KP, KØ, P-AA, RK, SB	JBT, KH, KP, SB

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With my signature I consent that the above listed articles where I am a co-author can be a part of the PhD thesis of the PhD candidate.



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# 1 Introduction

## 1.1 Population divergence and natural selection

Understanding the factors and mechanisms that initiate divergence and develop reproductive isolation between populations is a major quest in evolutionary biology. Natural selection has been forwarded as the most important force in creating differentiation in local populations (Barrett and Schluter, 2008), especially during the incipient stages of ecological speciation (Feder et al., 2012), but the mechanisms by which it modulates pheno- and genotypic frequencies in the divergence process is still not fully understood. Adaptive radiation is the evolution of ecological and phenotypical diversity, in which a lineage rapidly diversifies into the new lineages adapted to divergent environments (Schluter, 2000). Although evolution depends on the combined effects of natural selection, random genetic drift, mutations, and gene flow, where non-adaptive processes and correlated traits may play important role in changing gene frequencies of populations in pheno- and genotypic differentiation, it is only (divergent) natural selection that acts as a driving force for local adaptation in rapidly diversifying lineages (Schluter, 2000).

Parallel evolution describes the formation of a similar trait (or traits) through the same genetic and developmental pathways among distinct populations exposed to similar selection pressure (Wood et al., 2005, Haenel et al., 2019). If this similarity of trait(s) originates from different backgrounds in different populations, it is called convergent evolution. But even when populations are facing presumably similar selection pressures, parallel evolution may not emerge, as even small differences in the direction of selection can largely hinder parallelism (Thompson et al., 2019). Also, two isolated populations that originate from a common ancestral population can differ in their allelic composition (without mutation or migration) as a result of random genetic drift. If these alleles differ between populations at a quantitative trait locus, the populations may also differ phenotypically. But, two phenotypically similar populations may also share common alleles at quantitative trait loci, but otherwise display high marker distance between populations (Burstin and Charcosset, 1997). Therefore, it is important to consider biotic and abiotic conditions, as well as genetic and phenotypic diversity when studying the local adaptation and divergence.

Throughout the history of population genetics, various parameters and statistics have been developed to estimate the population differentiation. One of the best known is the fixation index  $F_{ST}$ , based on F-statistics, that have been used for many decades (Wright, 1951). It measures population differentiation due to genetic structure, expressed as values from 0 to 1, where a value of 1 implies that populations are completely separated and do not share any genetic diversity. Originally,  $F_{ST}$  was

defined to measure genetic distance using biallelic markers, but Nei (1973) introduced  $G_{ST}$  distance generalization, that uses heterozygosity within populations, to apply for multiple alleles. Both of these measurements are sensitive to mutation and decrease when the mutation rate of the genetic material is high. However, if the mutation rate is small relative to the migration rate,  $G_{ST}$  is not affected by mutations, and serves as a good estimate of the true differentiation (Whitlock, 2011, Verity and Nichols, 2014). In addition, when using markers that have high mutation rate, one can improve the power by comparing  $G_{ST}$  with Jost's  $D$  (Jost, 2008), as markers where  $G_{ST}$  underestimates divergence should have significantly elevated values of  $D$ . By studying the pairwise  $G_{ST}$  comparisons it is possible to assess for example spatial patterns of genetic differentiation, and if the genetic differentiation is correlating with the geographic distance. Typically,  $G_{ST}$  increases with the geographical distance, as populations that are further apart are expected to be less related due to reproductive barriers, and possible subject to drift based divergence.

## 1.2 Standing genetic variation

Except for purely plastic changes, adaptation to an environmental change or range expansion can arise either through selection on new beneficial mutations, or through selection from already existing standing genetic variation. Adaptation from standing genetic variation has been hypothesized to occur faster than from new beneficial mutations, because all the necessary alleles for expressing the needed phenotype are available immediately after changes within the environment (Barrett and Schluter, 2008). Standing genetic variation also offer beneficial alleles with higher population frequencies than mutations, which reduce the time needed to produce the locally adapted phenotype (Hermisson and Pennings, 2005). The evolution from standing genetic variation stands in contrast to adaptation from mutations, where evolution would have to wait for beneficial alleles to arise randomly, and thereafter distribute these alleles throughout the evolving population. Hence, the rapid evolution (<10 kyr) observed frequently in many northern post-glacial systems and in biological invasions may primarily be based on the standing genetic variation of the founder population (Jonsson and Jonsson, 2001, Præbel et al., 2013).

The amount of standing genetic variation sets the frame for selection and divergence process. But little is known how much variation is eventually needed for selection to work with. Furthermore, selection may also work actively to increase genetic variation, even in the case of a strong founder effect and minimal genetic variation (Labonne et al., 2016). Thus, reduced genetic variation does not necessarily prevent adaptation, as small populations may retain their adaptive potential (Wood et al., 2015). Also environmental heterogeneity has shown to affect the levels of variation (Huang et al., 2014). However, when selection is operating with smaller degree of genetic variation, it may lead to a

longer time for niche differentiation, adaptation, and ecological speciation (Gavrilets, 2004, Hendry, 2009).

The amount of genetic variation vary across the genome, where the location of regions with elevated or reduced variation may be paramount for the capability and pace of individuals and populations to adapt to novel environments and ecological niches (Rockman et al., 2010, Charlesworth and Campos, 2014, Reid et al., 2017). But little is known about the influence of the genome-wide distribution of variation in local adaptation. Evolution in the number of lateral plates between freshwater stickleback populations is considered as a classic example of rapid phenotypic adaptation from standing genetic variation, where the loci contributing to the phenotypic divergence have been detected (Colosimo et al., 2005). But standing genetic variation can also be neutral with no direct effect to the phenotype. Variation at neutral loci may not reflect the variation in loci contributing to the phenotype, and the linkage between a neutral loci and quantitative trait loci is, thus, very important when comparing neutral genetic and phenotypic variation. For example, previous studies have shown correlation between marker heterozygosity and fitness, also when using presumably neutral markers like microsatellites (Coulson et al., 1998, Coltman et al., 1999, Chapman et al., 2009). Selective sweeps may cause hitchhiking of neutral alleles due to linkage to an allele under selection (McVean, 2007). Without this linkage association, the relationship between genotypic and phenotypic distance becomes erratic. Studies of *Heliconius* butterflies have shown that genomic divergence increases with ecological and reproductive isolation, not only across the locus responsible for adaptive variation, but also at genomic regions unlinked to it (Supple et al., 2015). Similar pattern have observed in lake whitefish (*Coregonus clupeaformis*), when comparing the mean genetic differentiation between markers associated with quantitative trait loci, and those that are not associated (Gagnaire et al., 2013).

Adaptation from standing genetic variation is expected to slow down allopatric speciation under parallel selection, and facilitate progress toward sympatric speciation under divergent selection, but the extent of parallelism depends on the angle of divergence, i.e. the similarity of adaptation optimum (Thompson et al., 2019). Therefore, when divergence originates from standing genetic variation, we are expected to observe parallel pattern of genetic differentiation across populations adapted to the similar habitats, whereas in the case of divergent selection, adaptation from standing variation becomes less beneficial for hybrid fitness than adaptation from new mutation alone, because large pleiotropic effects of alleles are causing maladaptive phenotypes (Thompson et al., 2019). As genetic differences do not necessarily reflect phenotypic differences (Reed and Frankham, 2001, Moss et al., 2003) and there may be multiple developmental pathways leading to the same phenotype



(Arendt and Reznick, 2008), convergent evolution may play a crucial part in the divergence process and observed phenotypic parallelism.

Laporte et al. (2015) studied phenotypic parallelism of body shape in lake whitefish and discovered that both genetic parallelism, and multiple genetic routes contribute to parallel phenotypic evolution in the shape of fish. Similar patterns of convergent evolution have also been observed for example in adaptation to resident and anadromous life strategies (Perrier et al., 2013) and domestication in Atlantic salmon (*Salmo salar*) (López et al., 2019), morphological traits in cichlids (*Amphilophus citrinellus* species complex) (Elmer et al., 2014), hypoxia tolerance in sculpins (*Cottoidea* superfamily) (Mandic et al., 2018), and freshwater adaptation in three-spined sticklebacks (*Gasterosteus aculeatus*) (Pujolar et al., 2017). In other words, there may be multiple genetic routes to one phenotype. In addition, changes in the environment may cause changes in the organisms' phenotypes. This response to the environmental differences is caused by phenotypic plasticity, which is the ability of a single genotype to develop multiple phenotypes (West-Eberhard, 1989). Arctic charr is a classic example of species-complex displaying morphological diversity (Skoglund et al., 2015), and high degree of phenotypic plasticity in morphological traits (Klemetsen, 2010, Kristjánsson et al., 2018). Through plasticity, a population may adapt rapidly to the fluctuating environment without genetic changes, but also the amount of plasticity is a heritable trait. Therefore, the evolvability of a population is anchored in the amount of standing genetic variation.

### 1.3 Barriers to gene flow

At the early stage of a divergence, when genetic differentiation is very low or absent, formation of barriers to the gene flow is critical for the process, as gene flow works against segregation (Morjan and Rieseberg, 2004). Therefore, substantial amount of gene flow between populations may slow down or prevent the formation of discrete populations (Slatkin, 1985, Nagylaki and Lou, 2008, Blanquart et al., 2012). Barriers against gene flow can be categorised into pre- and postzygotic barriers, depending on whether the barrier acts before or after the reproductive event, and into in- and extrinsic barriers, depending on whether it is a biological or environmental barrier, respectively. Intrinsic barriers to gene flow include i.e. genome incompatibility, sexual selection, and assortative mating, whereas possible differences in spawning time or place are extrinsic barriers. In incipient sympatric speciation, with no physical boundaries between the diverging populations, differences in ecological niches may facilitate divergence as populations are exposed to different environments. Thus, hybrids may suffer a reduced fitness both because they fall between ecological niches, and as a result of intrinsic genetic incompatibilities between the parental genomes (Rundle and Whitlock, 2001). Indeed, Rogers and Bernatchez (2007) demonstrated how both intrinsic and extrinsic post-zygotic isolation mechanisms

works as progenies of hybrid backcross families of dwarf and normal lake whitefish either died during development, or hatched at a sub-optimal time. But in contrary to European whitefish (*Coregonus lavaretus L.*), lake whitefish morphs originate from a secondary contact, which may have had affect to the accumulation of genomic incompatibilities between morphs. In addition, European whitefish morph have observed to hybridise with highly specialized zooplanktovore vendace (*Coregonus albula*), indicating the lack of complete intrinsic isolation barriers (Kahilainen et al., 2011b). Thus, pre-mating barriers may play a crucial role in the divergence process. Size based assortative mating have been described in many fish species like coral reef cardinalfish (*Sphaeramia nematoptera*) (Rueger et al., 2016), sticklebacks (Rowland, 1989, Kraak and Bakker, 1998, Vines Timothy and Schluter, 2006), and sockeye salmon (*Oncorhynchus nerka*) (Foote, 1988). In European whitefish, size-assortative mating has been suggested to be an important mechanism for maintaining reproductive isolation between morphs despite high gene flow (Bhat et al., 2014, Svärdsön, 1979), but the development of reproductive isolation at the early stage of divergence has not been studied before. In the case of sympatric speciation, morphs are adapted to environmental differences and inhabit different niches. Due to this, similar individuals are more likely to encounter each other, therefore assortative mating may also be an artefact of spatial heterogeneity (Snowberg and Bolnick, 2012). Other mechanisms contributing to the forming of extrinsic barriers may be microhabitat segregation of spawning sites (Schneider, 2000), and diet based assortative mating that is shown to occur even within phenotypically unimodal populations (Snowberg and Bolnick, 2008).

## 1.4 The study system

The postglacial lakes in Fennoscandia are relatively young (< 15 kyr) and represent discrete and partly isolated environments, making them outstanding “natural laboratories” for studying evolutionary processes. This landscape of hundreds of lakes harbours a continuum of mono to polymorphic fish populations throughout the post-glacial landscape, and thus provides a good opportunity to study the factors and mechanisms that initiate and maintain population divergence and niche adaptation. Hence, this system allow for investigating evolutionary relevant questions, such as the extent of (non)parallel (i.e. parallel and nonparallel) evolution (*sensu* Bolnick et al., 2018) at large spatial scales, and also study temporal aspects of evolutionary diversification and adaptation due to the deglaciation history of the area.

European whitefish is a common fish species in postglacial lakes, especially in northern Fennoscandia. It is a polymorphic fish that has diverged into distinct morphs adapted to the three principal habitats (littoral, pelagic and profundal). The lakes are located in three watercourses, Pasvik River, Tana River, and Alta-Kautokeino River, that all drain into the Barents Sea. The lakes within these

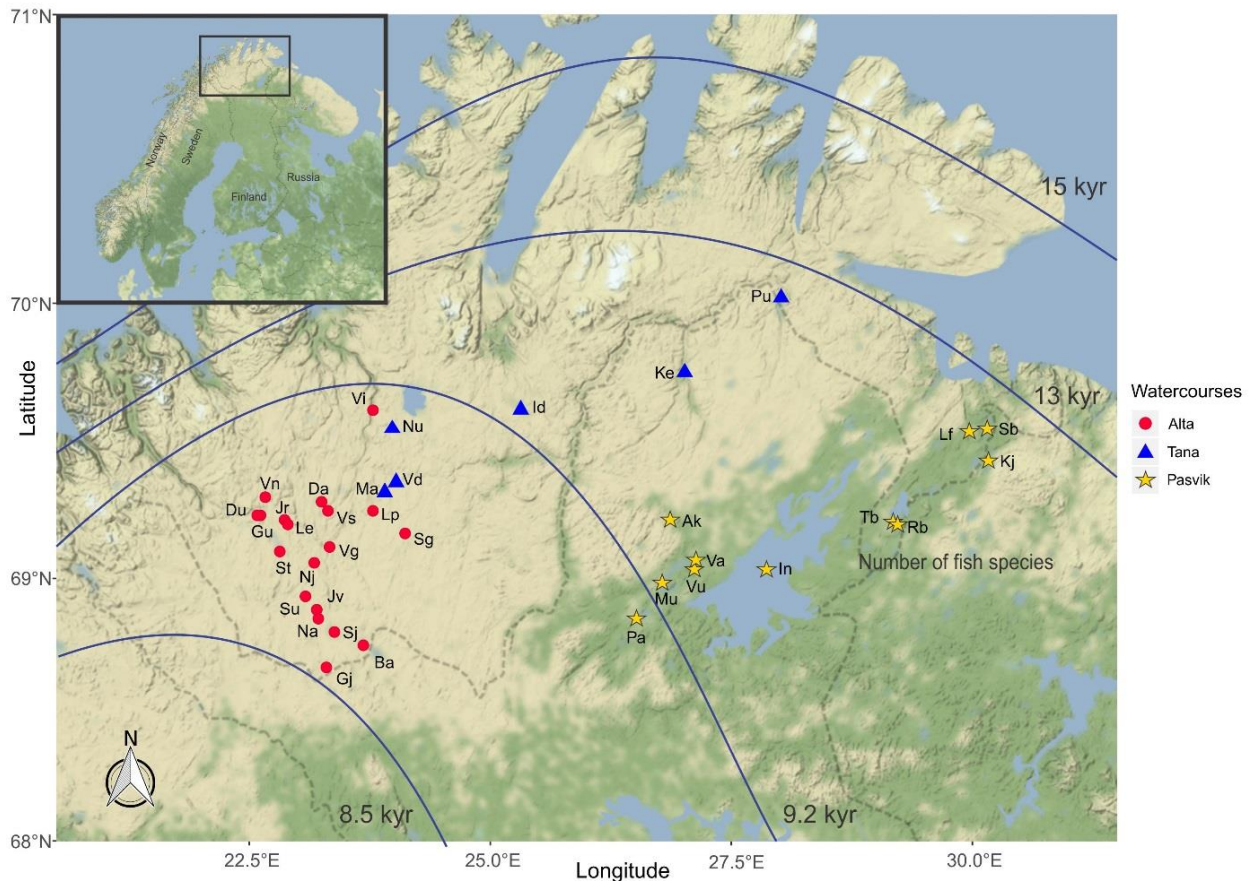


Figure 1. Map of the study area. Lakes in Alta, Tana and Pasvik watercourses are coded with red dots, blue triangles and yellow stars. Contour lines represent the retreating ice. Sampling sites are coded with abbreviations of lake names (see **Paper II**).

three watercourses are believed to have different geological ages, as the deglaciation occurred from the East towards the West (Sollid et al., 1973, Andersen and Borns, 1994, Kujansuu et al., 1998, Mangerud et al., 2004, Svendsen et al., 2004, Stokes et al., 2014, Stroeven et al., 2016). This suggests that the Pasvik watercourse was the first to be formed, followed by the Tana watercourse, and that the Alta watercourse was the last to be formed (Figure 1). When the lakes became ecological available, one clade of whitefish colonized the lakes from an eastern refuge, and thereafter diverged in sympatry (Østbye et al., 2005, Østbye et al., 2006, Præbel et al., 2013). In contrast to abundant monomorphic whitefish populations, polymorphic populations tend to be located close to the main stem of the rivers, where some of the lakes harbor up to four distinct morphs (Siwertsson et al., 2010, Couton, 2012, Kahilainen et al., 2014, Kahilainen et al., 2017).

Different morphs have been identified based on the body morphology and gill raker number, which are heritable and ecologically important traits (Svärdson, 1952, Svärdson, 1979, Bernatchez, 2004). The number of gill rakers is associated with diet composition (Kahilainen and Østbye, 2006, Østbye et al., 2006, Kahilainen et al., 2011a), where morphological differences in gill rakers affect the

feeding efficiently (Sanderson et al., 2001, Roesch et al., 2013). The large sparsely rakered (LSR) whitefish, occupying the littoral habitat is considered to be the ancestral morph that colonized these lakes (Østbye et al., 2005), and it is also the only morph with many allopatric populations throughout northern Fennoscandia (Siwertsson et al., 2010). The LSR whitefish mainly feeds on benthic macroinvertebrates and has the intermediate number of gill rakers compared to the other morphs. The densely rakered (DR) whitefish, with the highest number of gill rakers, occupy the pelagic habitat, and mainly feed on zooplankton. The small sparsely rakered (SSR) whitefish is specialized to forage on profundal benthic prey and have the lowest number of gill rakers (Kahilainen and Østbye, 2006, Harrod et al., 2010). In some lakes in the Pasvik watercourse, large bodied densely rakered (LDR) pelagic whitefish with high number of gill rakers use near surface pelagic and littoral habitats (Couton, 2012, Kahilainen et al., 2014, Kahilainen et al., 2017). In addition to the gill raker and habitat differences, whitefish morphs also differ in morphology and life history traits (Østbye et al., 2006, Harrod et al., 2010, Siwertsson et al., 2013a, Kahilainen et al., 2017), where a high number of gill rakers usually is associated with smaller body size and early maturation. The variation in the degree of both phenotypic and genetic divergence among the ecomorphs throughout northern Fennoscandia represents a speciation continuum at a landscape level, where most divergent polymorphic populations are found in the presumably oldest Pasvik watercourse, (Kahilainen and Østbye, 2006, Østbye et al., 2006, Siwertsson et al., 2010) - pinpointing the usefulness of this system to study evolutionary factors and processes.

Reproductive isolation between the sympatric whitefish morphs is relatively weak, as hybrids are occurring commonly in nature. For example, in some lakes in the Pasvik watercourse, the reproductive barriers have been observed to be weakened due to an invasion of a non-native species (Bhat et al., 2014). The invasive species, vendace, is superior competitor to the DR whitefish and has excluded the DR morph from its preferred pelagic habitat and into the littoral habitat (Bøhn et al., 2008). This secondary-contact of the LSR and DR morphs has created a situation of speciation reversal, where the genetically differentiated LSR and DR ecomorphs have merged together forming hybrid swarm (Bhat et al., 2014, Bhat, 2016). Although the different morphs are capable of reproducing with each other, the accumulation of genetic differentiation between the sympatric morphs suggest that there are forces driving them apart as subtle, but highly significant genetic and phenotypic differences between different morphs have been observed (Østbye et al., 2006, Præbel et al., 2013, Siwertsson et al., 2013b). But not much work (if any) has been done to illuminate isolation mechanisms among these morphs, especially at the landscape level. Previous studies have suggested that the repeated occurrence of sympatric morphs in the three main habitats is the outcome of divergent selection (Præbel et al., 2013), and the possibility of neutral divergence due to random genetic drift is considered

as an unlikely explanation for the phenotypically differentiated morphs. However, no previous studies have tested this hypothesis using a large landscape level dataset. There is number of convincing examples of speciation with gene flow (i.e. reviewed in Hey, 2006, Feder et al., 2012), but little is known about the formation of reproductive barriers between sympatric whitefish morphs in the early stage of speciation process. In the northern Fennoscandian lake systems climatic conditions also pose an effective hinder for studying the reproductive barriers *in situ*, as the lakes are normally covered with ice too thin to work on and too thick to boat through during the spawning season. From sporadic observations, it also appears that the timing and duration of the spawning times differ for different morphs and even among lakes in the area (Kahilainen et al., 2014. Pers. Com. Rune Knudsen, Eleonor Beck). Additionally, the location of spawning sites is still not known in most of the lakes and it is not known which morph(s) utilize these spawning grounds. But, despite these logistical hinders, the knowledge of likely types of reproductive barriers will provide crucial information for understanding the evolution of these whitefish morphs.

## 1.5 Objectives

European whitefish, with its many replicated populations at landscape level, is an important species for studying evolutionary processes. Knowledge about how divergence arises in sympatry under different levels of standing genetic variation and how reproductive isolation is formatted and maintained is essential for understanding speciation. The aim of this thesis was to investigate the interplay between various evolutionary processes and standing genetic variation in shaping phenotypic and genetic divergence in whitefish. Special emphasis was given to how alternative evolutionary forces of random drift and natural (divergent) selection participate in shaping phenotypic and genetic variation, consequently leading to population divergence via local adaptation, and to the role of standing genetic variation as a source of adaptive variation.

The main objectives addressed in this thesis are:

- I. Identify phenotypic traits that are important for the divergence process (local adaptation), test for interactions and signatures of divergent selection versus random genetic drift among traits and test the correlations with the environment (**Paper I**)
- II. Investigate the association between phenotypic and genotypic variation in a speciation continuum displaying alternative levels of standing genetic variation, and assess the

consequences of growing geographic distance to the genetic and phenotypic distances at the landscape level (**Paper II**)

- III. Examine prezygotic isolation mechanism as a driving factor of divergence between the three sympatric morphs (**Paper III**)

## 2 Materials and methods

The data for **Paper I** consisted of already collected phenotypic and genetic samples of whitefish morphs from nine lakes. We selected lakes where all three main habitats (littoral, pelagic and profundal) were present. In total, 999 whitefish were classified as DR, LSR and SSR morphs based on their morphology. The number of gill rakers were calculated under stereomicroscope and sex, maturity, and total length of fish was noted. Individuals were genotyped at 21 microsatellite loci for genetic analysis, from which 13 loci were used as neutral marker data to compare neutral genetic differentiation and observed quantitative genetic differentiation with the aim of differentiate random genetic drift from selection.

In **Paper II** 3430 fish were collected from 77 populations in 36 lakes harbouring mono or polymorphic whitefish populations. Most of the samples were collected previously. From each individual we measured total length and weight, number of gill rakers, morph assignment, age, which was estimated by counting the annual rings from otoliths and collected a fin clip for genetic analysis. Here, we used microsatellites from 19 loci as a proxy to standing genetic variation. Number of gill rakers, Fulton's condition factor, and standardised length at age were used as phenotypic traits when assessing if genotypic variation reflects the phenotypic variation across whitefish populations.

For **Paper III**, two lakes in the Alta watercourse were sampled in mid-October, just before the lakes were expected to freeze over. Fish were classified as DR, LSR and SSR whitefish in the field based on their morphology, and total weight and gonad weight were measured. From each individual we noted the sex, sexual maturity and total length. In addition, a fin tissue sample was collected for the genetic analysis, and first left branchial arch to calculate the number of gill rakers. We used gonadosomatic index (GSI) of 300 whitefish (139 males and 161 females), calculated separately for both sexes, to evaluate the degree of spawning readiness between the three morphs, and to investigate the possible pre-zygotic isolation mechanisms among the morphs.

### 3 Summary of the papers

#### Paper I

**Diversifying selection drives parallel evolution of gill raker number and body size along the speciation continuum of European whitefish. *Ecology and Evolution* (2018), 8 (5), 2617 - 2631.**

Comparison of expected divergence under random genetic drift and observed divergence in phenotypic traits in European whitefish revealed that number of gill rakers and the length of fish have been under diversifying selection, and that the evolution of these morphs has been driven by isolation through habitat adaptations. Signals of selection were detected for most populations and were most pronounced for the divergence of the DR and the SSR whitefish. However, the whitefish morphs in Alta and Tana appeared to be subjected to more genetic drift than the whitefish morphs in the Pasvik watercourse. The relationship between number of gill rakers and length was antagonistic, suggesting that a higher number of gill rakers tended to be correlated with smaller size of fish. In addition, the mean lengths of all three studied morphs were greater in the Alta watercourse than the Pasvik watercourse. We concluded that diversifying selection acting on gill raker number and body size has played a significant role in the ongoing adaptive radiation of European whitefish morphs in this region.

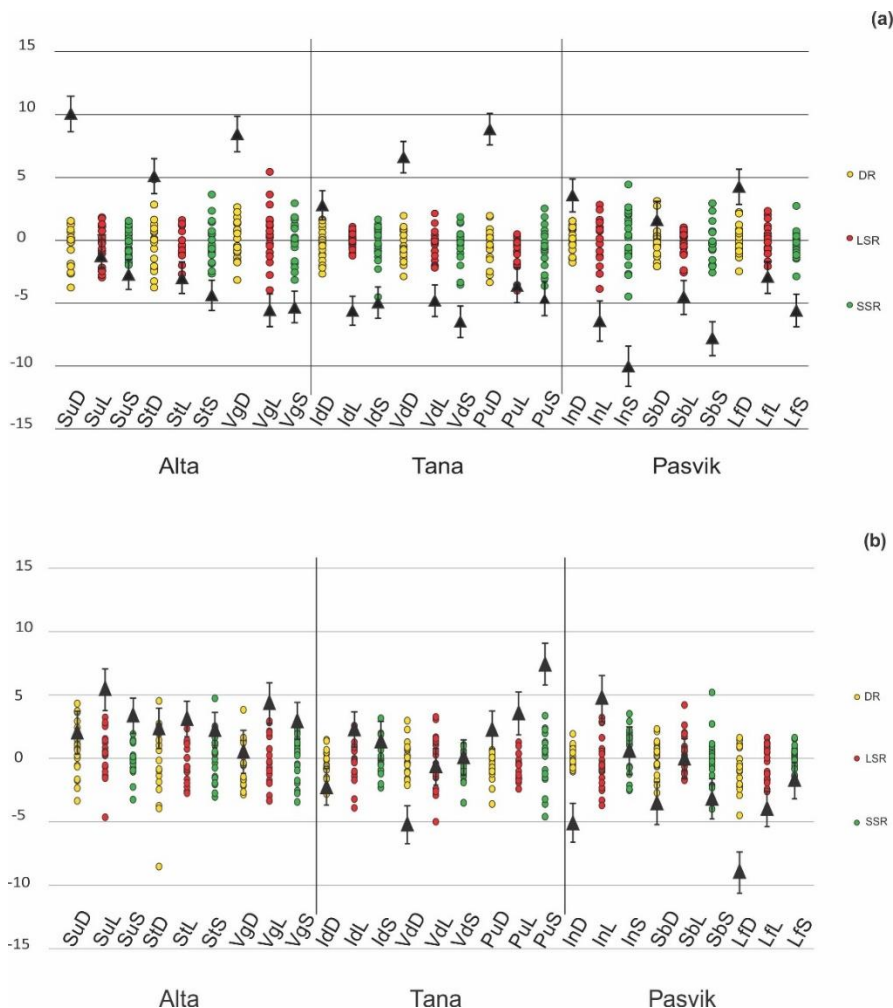


Figure 2. Population means in gill raker (a) and total length (b) traits. Triangular dots with error bars represent population means from the real data with 95% credibility interval. Small dots represent the amount of variation expected under drift-based divergence. Zero level is the ancestral mean. In this picture, there are 20 simulated replicates representing different scenarios.

## Paper II

### (Non)parallel patterns of standing genetic variation and phenotypic diversity in a speciation continuum of European whitefish in northern Fennoscandia: manuscript

This paper elucidates how the evolution of morphs and adaptive phenotypic traits have proceeded under alternative levels of standing genetic variation (SGV) in an ongoing adaptive radiation in European whitefish at a landscape wide scale. Both heterozygosity and number of alleles decreased from the older Pasvik watercourse towards the youngest Alta watercourse. Despite this drastic decrease in SGV, the level of phenotypic differentiation varied across the studied landscape, and thus represented a gradient of phenotypic variation from small to large phenotypic differences. Whitefish clustered genetically according their original watercourse, and genetic divergence increased with geographic distance, suggesting isolation by distance. The landscape wide phenotypic divergence was not as affected with the increasing geographic distance as the genetic divergence. However, when comparing sympatric morphs within each lake, large genetic distances were associated with large phenotypic distances, whereas small genetic distances were associated with both small and large phenotypic distances. We therefore concluded that the evolution of European whitefish morphs is an intricate case of parallel and non-parallel speciation of phenotypes, where the allelic variants underlying adaptive traits may be present or new combinations have been evented at the different levels of available standing genetic variation.

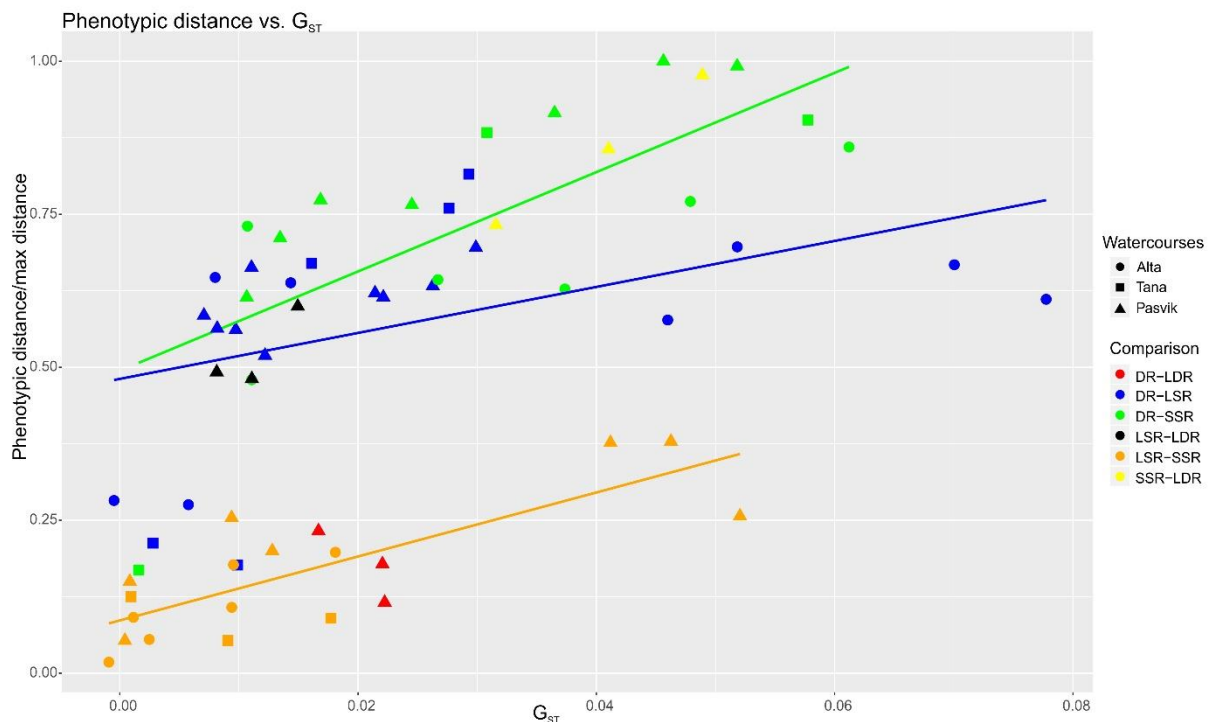


Figure 3. Pairwise comparison of  $G_{ST}$  and phenotypic distance between eco-morphs in each lake among watercourses. Different shapes represent the three watercourses, and morph wise comparisons in each lake are color-coded accordingly. Straight lines represents the regression of each eco-morph pair



comparison (DR-LSR:  $R^2 = 0.21$ ,  $P = 0.032$ , DR-SSR:  $R^2 = 0.53$ ,  $P = 0.001$ , LSR-SSR:  $R^2 = 0.65$ ,  $P < 0.001$  and the overall regression across all comparisons  $R^2 = 0.34$ ,  $P < 0.001$ ). As LDR is present only in three lakes, regression lines are not presented for LDR comparisons.

### Paper III

#### Allochrony as a potential driver for reproductive isolation in adaptive radiations of European whitefish ecomorphs: Early view: Ecology of Freshwater Fish (2019), doi: 10.1111/eff.12486.

The different isolation mechanisms driving the ongoing divergence in adaptive radiation of whitefish in northern lake systems are still not fully understood. As the morphs mainly share the same spawning ground(s) in the littoral zone, differences in spawning time may contribute to the formation of reproductive isolation between them. By using gonadosomatic index (GSI) as a proxy to investigate this pre-zygotic isolation mechanism, the three whitefish morphs were found to differ in GSI-values within and between both study lakes, giving support to the hypothesis of spawning time differences of the morphs. Pairwise  $F_{ST}$  values between morphs were small, but highly statistically significant, suggesting that some reproductive barriers have formed among the morphs. In addition, the study revealed possible sex-specific route for gene flow between morphs as males of one morph had equal onset of maturity as another morph, giving novel insights into the ongoing gene flow observed between morphs. The result supports allochrony as a driver for the divergence process of whitefish morphs, but the possibility that differences on spawning grounds may also contribute to the reproductive divergence between morphs cannot be ruled out.

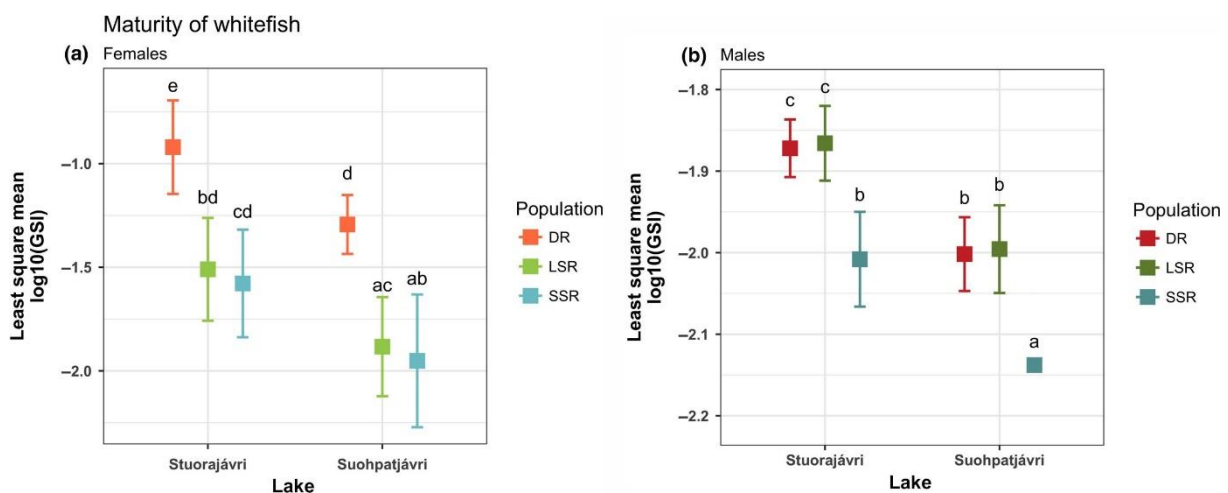


Figure 4. Plots showing the differences in maturity of the whitefish ecomorphs, for females (a) and males (b) and across two lakes. This was done using least square means for the specified factors in the linear model, and p-values were adjusted for multiple comparisons by Tukey's HSD. Boxes indicate the least square mean of  $\log_{10}(\text{GSI})$ , and error bars indicate the 95% confidence interval of the least square mean. Means sharing a letter have no statistically significant difference at the alpha level 0.05.

## 4 Discussion

In this thesis, it was demonstrated how selection has shaped the whitefish population across a large spatial scale despite the clear effect of random genetic drift - presumably arising from the colonization history (**Paper I**). The diversification of European whitefish follows a (non)parallel trajectory, as morphs in the different watercourses were characterised with various levels of phenotypic and genetic divergence (**Paper I, II**). Here, large genetic divergence among sympatric whitefish morphs were accompanied with the largest phenotypic trait divergence, whereas small genetic differentiation was related to both small and large phenotypic trait divergence in the study lakes. However, the association between phenotypic and genetic variation was more ambiguous when comparing the populations across the landscape to the presumably older population in Pasvik watercourse. This suggested that the divergence may emerge at alternative level of genetic variation leading to (some extent) parallel phenotypic evolution despite the signature of isolation by distance in genetic markers. As the amount of genetic variation differs between watercourses, emergence of the whitefish morphs could be result of convergent evolution, where the same outcome is achieved with multiple genetic routes. The positive correlation between marker heterozygosity and condition, representing the fitness of the individual fish, was weak, but statistically significant, and was only observed in the Alta watercourse, implying watercourse specific differences in phenotypic and genetic traits (**Paper II**). Nevertheless, the three main morphs were recognised in each of the contemporary watercourses, revealing similar pattern of habitat specialised morphs across the landscape. The observed diversification has been hypothesized to be an outcome of parallel evolution that have happened separately in each lake. Since the formation of reproductive barriers between sympatric populations is crucial for divergence, the contribution of allochrony to the extrinsic pre-zygotic isolation mechanism and formation of reproductive isolation was assessed by comparing the spawning readiness between morphs (**Paper III**). As the morphs are adapted to their specific habitat (**Paper I**), they experience differences in the environment, such as water temperature, light, and prey availability. This has shown to affect the gonad development and maturation. We observed, as hypothesized, differences in the state of maturity between morphs and concluded that allochrony has likely been building up the reproductive isolation between sympatric whitefish morphs.

My thesis evaluated the association between standing genetic variation and phenotypic variation, investigated if the phenotypic differentiation of the three morphs is a result of selection, or originates from random genetic drift, and studied how reproductive isolation may builds up between differentiating populations.

In the following sections, I will discuss the main findings of this dissertation in relation to historical contingency, adaptive radiation, and speciation.

## 4.1 Patterns of phenotypic and genotypic divergence in northern Fennoscandian whitefish

We observed subtle phenotypic differences between and within watercourses (**Paper I, II, III**). The phenotypic differences among morphs between the three watercourses changed gradually, representing a continuum of populations from phenotypically most divergent populations in the Pasvik watercourse to the least divergent populations in the Alta watercourse. In the latter, the morphs were characterized with an overall larger body size, and better condition factor compared to the fish in older watercourses (**Paper I, II**). As adaptive phenotypes are shaped by selection, evolutionary time may have been an important contribution to the differences in degree of phenotypic divergence of whitefish morphs within lakes among the watercourses. Post-glacial lakes in northern Fennoscandia originates from the previous deglaciation, when the Fennoscandian ice-sheet retreated from northwestern Russia towards the southwest Scandinavian mountains, forming the three main contemporary watercourses (Sollid et al., 1973, Andersen and Borns, 1994, Kujansuu et al., 1998, Mangerud et al., 2004, Svendsen et al., 2004, Stokes et al., 2014, Stroeve et al., 2016). Based on the assumed colonization time and order, whitefish populations in the Alta watercourse are expected to be 5000 years (~1000 whitefish generation) younger than whitefish populations in lakes in the Tana and Pasvik watercourses (Østbye et al., 2005, Præbel et al., 2013, Præbel et al. in prep). Therefore, it may be hypothesized that the more subtle phenotypic divergence observed among the whitefish morphs in the Alta watercourse may be a consequence of shorter evolutionary diversification time. But also other factors, like differences in selection pressures between watercourses, micro and macro-ecological differences among lakes, and local environmental and climatic differences among lakes may have contributed to the observed divergence (Nosil et al., 2009).

The existence of morphologically differentiated populations in postglacial lakes is usually associated with ecological opportunity, resource polymorphism, and adaptation (Schluter and Rambaut, 1996, Skúlason et al., 1999), where populations are often, but not always, expressing genetic differences. The pairwise  $G_{ST}$  values among whitefish morphs and populations ranged from zero to 0.22, and the genetic distance between populations increased with geographic distance from the Pasvik watercourse towards the Alta watercourse, suggesting that random drift may have affected to the genetic divergence across the landscape. A similar pattern of isolation by distance (IBD) – or by colonization (IBC), has been frequently found in many other species, such as birds, fruit bats, and coral

reef animals (Storz, 2002, Selkoe et al., 2014, Spurgin et al., 2014), and has also been observed in post-glacial freshwater fish populations (Laikre et al., 2005, but see also Shikano et al., 2015), as well as among whitefish populations in Denmark (Hansen et al., 1999, Bradbury and Bentzen, 2007). Reduction in genetic variation across landscape is common phenomenon after post-glacial recolonization (Hewitt, 1996, 1999), but both colonization history and ongoing gene flow contributes to the current patterns of genetic structure within population (Shaw et al., 1991, Angers et al., 1999, Costello et al., 2003, Caldera and Bolnick, 2008, Raeymaekers et al., 2008). As Pasvik, Tana, and Alta watercourses are not connected, recent migration between the three watercourses has not likely happened after the formation of these watercourses. The findings in **Paper I** provide support to this lack of interpopulation coancestry and gene flow between different watercourses, but also suggested that whitefish morphs in Alta and Tana watercourses have been subjected to more genetic drift than whitefish morphs in Pasvik watercourse. Random genetic drift may have larger effect to the number of alleles in a population than to expected heterozygosity, as rare alleles are more likely to be lost in bottlenecks, while expected heterozygosity is not that sensitive to possible bottlenecks (Allendorf, 1986). In addition, bottlenecks may reduce neutral genetic variation more rapidly than genetic variation for quantitative traits (Lande, 1988). But as the results obtained from the microsatellite data are in consensus with results from a genome wide SNP dataset obtained from RAD-sequencing of whitefish from the same lakes (Præbel et al. in prep), it is likely that the neutral variation is a good indicator for the standing genetic variation of whitefish in these lakes. There was significant reduction of both allelic richness and heterozygosity when moving from the Pasvik watercourse towards the Alta watercourse, where allelic richness was just a subset of the allelic richness present in the Pasvik watercourse (**Paper I, II**). Within each watercourse, whitefish had approximately similar standing genetic variation, which suggest that each of the watercourses was colonized at single events in evolutionary time, and that there has not been contemporary migration among watercourses. However, the dendrogram illustration of pairwise genetic  $G_{ST}$  and  $D$  comparisons placed the whitefish population in Aksujärvi lake, that is a lake within the Pasvik watercourse, in a same branch with the lakes in Tana watercourse (Figure 5), suggesting that this lake may have shared connection to the Tana watercourse. This is supported by the physical location of Aksujärvi within the Pasvik watercourse, as Aksujärvi is located closest to the Tana watercourse of all sampled lakes within Pasvik watercourse. The single colonization event in each watercourse was also supported by the genetic clustering across watercourses, where whitefish within watercourses were genetically more similar to each other, than when comparing whitefish across watercourses (**Paper I, II**). In addition, Østbye et al. (2006) studied a smaller subset of whitefish populations from these watercourses and using six microsatellites they found a similar pattern. Thus, it appears that the whitefish populations within watercourses follow a similar evolutionary trajectory, where standing genetic variation brought in at the event of colonization

and watercourse isolation fuel genotypic and phenotypic (non)parallel divergence. This also suggests that the occurrence of similar ecological traits and phenotypes among whitefish morphs across the three watercourses, that are displaying decreasing amount of genetic variation, is the product of convergent evolution.

Signatures of both within and between lakes divergence was observed in all studies conducted within this thesis (**Paper I, II, III**). The different morphs clustered partially together across their original lakes, but especially the DR morph populations were oftentimes located together within their original watercourse (**Paper I**, Figure 5). This may refer to diversifying selection and parallel evolution of morphs in the same direction, as similarity of morphs in different lakes is expected under similar selection pressures (Schluter, 2000), but the possibility of parallel colonization cannot be ruled out. As the colonization of these watercourses took thousands of years, the most prominent divergence between LSR and DR whitefish may have already happened before whitefish colonized Alta watercourse, i.e. the question whether the morphs in Alta are the outcome of parallel colonization, instead of parallel evolution, remains unanswered.

Overall, the results revealed a continuum of both genotypic and phenotypic differentiation across the landscape. The results also imply that a thorough analysis of the demographics of the postglacial recolonization is needed to fully elucidate the evolutionary trajectory of this continuum of divergence.

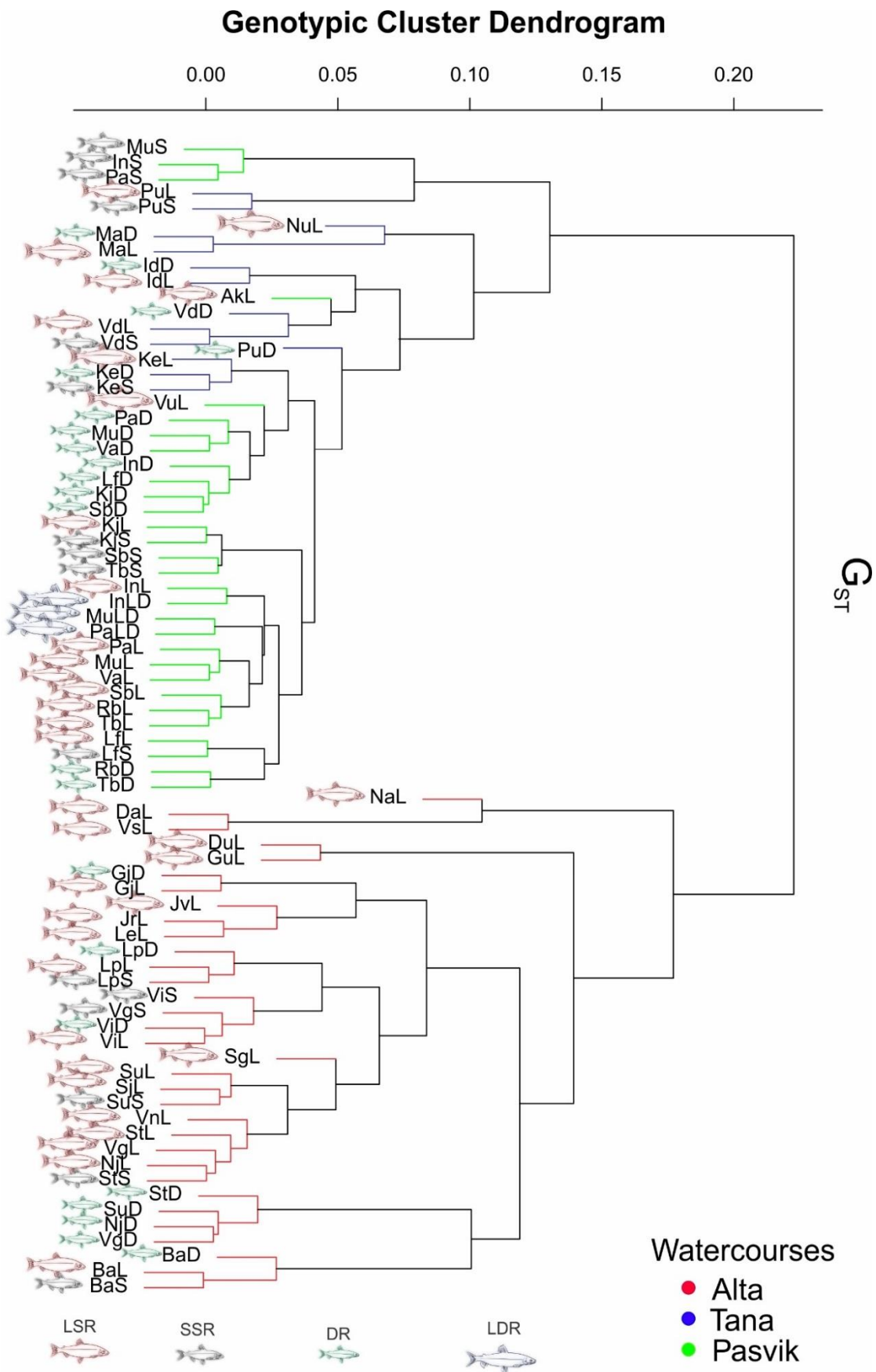


Figure 5. Dendrogram illustration of the pairwise  $G_{ST}$  between morphs in study lakes. For population abbreviations, see **Paper**

## 4.2 Signals of random genetic drift or natural selection?

Phenotypic parallelism across similar habitats may be used as a support to adaptation and natural selection, but neutral divergence based on random genetic drift cannot be ruled out based on the patterns of spatial variation in phenotypic traits (Runemark et al., 2010). Therefore, it is important to test whether the observed divergence could have been achieved through neutral divergence. The multitude of lake replicates in northern Fennoscandia offers an excellent opportunity to investigate the effect of drift and selection, and how these forces have contributed to the phenotypic and genetic divergence of sympatric whitefish populations (**Paper I**).

The rate of genetic drift in a population is directly dependent on the effective population size (Wright, 1931). During the founder events originating from the colonization history of Fennoscandian lakes, only subset of the original population presumably colonised new areas. Therefore potential non-adaptive divergence has been an important factor in the divergence process of whitefish morphs across this area, as the repeated bottlenecks forms a spatial correspondence to the random genetic drift (Slatkin and Excoffier, 2012). Indeed, our results suggest that the whitefish populations in the Alta and Tana watercourses have been subjected to more genetic drift than the whitefish from the Pasvik watercourse, likely originating from the colonization history (**Paper I**). This was evident as genetic distance between populations increased with the geographic distance when moving from north-east towards to the western populations (**Paper II**). Hence, the demographic processes have highly influenced genetic variation of whitefish in these lakes, likely restricting the genetic material available for adaptation, because the ability to evolve and adapt may be related to both heterozygosity and the number of alleles present within a population (Allendorf, 1986, Frankham et al., 2014). Although the populations in Alta watercourse had substantially reduced amount of standing genetic variation, and the phenotypic divergence was more subtle compared to the populations in older watercourses, the signals of selection were obvious, as the observed divergence would not have been achievable through neutral divergence based solely to the random drift. In addition, the different morphs were more adapted to the corresponding habitat than what would have been expected based on their shared phylogenetic history (**Paper I, II**). Therefore, the appearance of the morphs, combined with phenotype-environment correlation, seems to be the outcome of adaptation, where diversifying selection has played a significant role by shaping the gill raker number and body size of European whitefish morphs in this region despite the varying amount of drift (**Paper I**). But even when adaptation to the similar selective environments may contribute to the parallelism in phenotypic responses across the landscape, the magnitude of these responses may be species specific, and have various genetic backgrounds (Raeymaekers et al., 2017).

### 4.3 Are the levels of genetic and phenotypic variation related?

Whitefish populations throughout the study area represent a speciation continuum where both genetic variation and phenotypic divergence are largest in the easternmost, and oldest, Pasvik watercourse, and gradually decreasing towards west and Alta watercourse (**Paper I, II**). When comparing morphs within each lake across the landscape, larger genetic differences were accompanied with larger phenotypic differences. Similar results, using genome-wide single-nucleotide polymorphisms, have been observed in sympatric lake whitefish species pairs (Gagnaire et al., 2013). But, when comparing DR morph to the LSR and SSR morphs, small genetic distances were not necessarily related to small phenotypic distances (**Paper II**). As gene flow is known to slow down or prevent diversification, whereas reproductive isolation reinforces it, the assumption that larger genetic differentiation is also associated with more profound phenotypic differences in sympatric populations is justified. Furthermore, the comparison of genetic versus phenotypic distance suggest that low genetic divergence may not prevent phenotypic divergence. Indication of this have been seen in Atlantic salmon (*Salmo salar*), where sympatric populations in Teno river display low level of genetic differentiation, but marked differences in the distribution of life history strategies (Aykanat et al., 2015).

A positive, and statistically significant, correlation was observed between marker heterozygosity and a fitness trait (condition factor) only in the Alta watercourse (**Paper II**). The high mutation rate of microsatellites is thought to increase the decay of linkage disequilibrium (Pogson and Zouros, 1994, Thelen and Allendorf, 2001), which may explain the lack of correlation in the oldest Pasvik watercourse populations, whereas populations that have undergone bottleneck or founder events, are expected to experience linkage disequilibria due to drift (Hansson and Westerberg, 2002). Combined, this suggests that the younger populations which have experienced a prominent amount of drift, like in the Alta watercourse, may show stronger linkage disequilibria. Additionally, as populations may diverge despite of gene flow under selection (Nosil, 2008), lack of complete correspondence between phenotype and molecular markers is expected when reproductive isolation is incomplete (Winker, 2009, Patten, 2010).

### 4.4 What contributes to the formation of reproductive isolation?

A reproductive barrier may be considered important if it is a strong inhibitor to gene flow alone (Coyne and Orr, 2004). But the strength of different barriers, and how they contribute to the total reproductive isolation over organism life cycle may vary, as barriers that operate later are preventing gene flow that has escaped earlier-acting barriers (Coyne and Orr, 1989, 1997, Ramsey et al., 2003). Therefore those



that act earlier in the life cycle (i.e. prezygotic barriers) may contribute more than later-acting barriers to total reproductive isolation, even when later acting barriers may be stronger when acting alone (Schemske, 2000, Ramsey et al., 2003, Nosil et al., 2005, Schluter, 2009).

In European whitefish hybrids occur frequently between the DR and LSR, as well as LSR and SSR morphs (Bhat et al., 2014, Præbel et al. unpublished) which may refer relatively weak postzygotic reproductive isolation between morphs (**Paper I, II**). This is not surprising, due to the young age of this adaptive radiation (Funk et al., 2006). Therefore, the divergent selection driving the morphs apart towards niche specialisation and adaptation must be strong to overcome the effect of gene flow, otherwise the cumulative effect of isolation barriers may not be enough to keep the populations separated, and divergence will be lost via introgression (Wright, 1931, Nosil, 2008, Feder et al., 2012). When species diverge and adapt to a specific niche, they encounter different environment conditions. Environmental factors, like water temperature and prey availability, have been shown to be important factors determining size at sexual maturity, somatic growth, and gonad development in whitefish (Kahilainen et al., 2003, Hayden et al., 2014), and have contributed to the maturation timing in salmonids (Hendry and Day, 2005). Consequently, allochrony, together with spatial differences in spawning, may reinforces the ecological divergence, and help to build up barriers to gene flow between the whitefish morphs. Kahilainen et al. (2014) suggested that the DR morph is the first one to spawn, followed by the LSR and LDR morphs, and thereafter the SSR morph, as the last morph to spawn. The advancement of the maturity, as well as the proportion of mature individuals per morphs followed the same pattern supporting previous field observations (**Paper III**). In addition, we observed sex specific differences in the advancement of maturity. In general, DR whitefish were further advanced in sexual maturity than LSR whitefish, which in turn were more mature than SSR whitefish. However, LSR males displayed a similar state of maturity to DR males, whereas female LSR and male and female SSR whitefish were less mature. The observation that the LSR males are mature at the same time as the DR whitefish, may suggest sex specific routes to the hybridization and gene flow between diverging morphs. As a consequence, sexual selection and assortative mating could well be important factors contributing to the maintaining of reproductive isolation between whitefish morphs. Cichlid fish study has shown that phenotypic differences, rather than genetic distance, promotes assortative mating between morphs in adaptive radiation (Stelkens and Seehausen, 2009). Because whitefish morphs differ in size, size assortative mating between different morphs may arise. Indeed, size based assortative mating has been observed in some salmonids, including whitefish (Svårdson, 1979, Foote, 1988). In addition, temporal differences in spawning time has often, but not always, observed between lake whitefish morph pairs (Fenderson, 1964, Chouinard et al., 1996). Prezygotic isolation may evolve between populations as a by-product of adaptation to environment differences (Schluter, 2001,

Rundle and Nosil, 2005, Vines Timothy and Schluter, 2006). Thus, allochrony has likely been important factor in the development of reproductive barriers between whitefish morphs in northern Fennoscandian lakes.

Studies of *Drosophila* species has shown that in sympatry, the prezygotic isolation evolves faster than postzygotic isolation, but both pre and postzygotic reproductive isolation increase with divergence time between taxa (Coyne and Orr, 1989, 1997). The same pattern of prezygotic isolations evolving well before postzygotic isolation has also been observed in several fish species, such as sunfishes and cichlids (Bolnick and Near, 2005, Stelkens et al., 2010). Furthermore, the studies on stickleback fish has found that young stickleback species pairs display prezygotic and environmentally based postzygotic isolation, while older pairs show both prezygotic and intrinsic postzygotic isolation (Schluter, 1993, Rundle et al., 2000, Kitano et al., 2007, Hendry et al., 2009). The possible postzygotic isolation mechanism in northern Fennoscandian lakes has not yet been studied, but studies of sympatric morph pairs have revealed intrinsic isolation originating from ecological divergence between dwarf and normal lake whitefish (Lu and Bernatchez, 1998, Rogers and Bernatchez, 2006).

## 4.5 Ecological speciation

The theory of ecological speciation predicts that the reproductive isolation between populations evolves as a by-product of ecologically based diversifying selection (Nosil, 2012). Speciation can be described as a continuum from continuous variation, within panmictic populations, to complete and irreversible reproductive isolation (Hendry, 2009). When moving along this continuum, populations are expected to experience partially discontinuous variation and minor reproductive isolation at the early state of speciation process. The observed divergence in European whitefish have been thought to be an outcome of diversifying selection and adaptation (Østbye et al., 2006, Harrod et al., 2010), but it was the present study that first time tested this hypothesis at a landscape wide scale (**Paper I**). The strong phenotype-environment correlation in the study lakes across three watercourses gave support to adaptive divergence. As the divergence increases with time due to ecological adaptation, populations are expected to experience decreasing amount of gene flow (Funk et al., 2006). We observed these signatures as the interpopulation relatedness, measured by coancestry coefficient, was smallest in the oldest Pasvik watercourse that harbours the most divergent morphs (**Paper I**). Furthermore, phenotypic trait distributions were oftentimes overlapping between morphs especially in the Alta watercourse that was presumably youngest of the three studied watercourses (**Paper I, II**). Nevertheless, phenotypic divergence increased with genetic divergence in all three watercourses (**Paper II**). When populations are experiencing ecological divergence, a positive correlation between

the degree of adaptive phenotypic divergence and levels of neutral genetic differentiation is expected, indicating that adaptive divergence restricts gene flow (Nosil et al., 2008).

As the reproductive isolation between whitefish morph is not complete, hybridisation between morphs occurs frequently. In the case of incipient ecological speciation, temporal differences in spawning time may be important in building up reproductive isolation between diversifying populations (Schluter, 2001). Indeed, allochrony seems to be working towards stronger reproductive isolation, reinforcing the ecologically based divergence between whitefish morphs (**Paper III**). But it is good to keep in mind that reproductive isolation alone does not cause speciation. Other factors, such as evolutionary force(s) (i.e. selection, random drift, or mutations) and ecological opportunity also must be present to create genetic differences between populations. Some theoretical models have suggested that both disruptive selection and assortative mating must be present, and fairly strong, before sympatric speciation may occur (Matessi et al., 2001, Bolnick, 2004, Bürger and Schneider, 2006, Doebeli et al., 2007). When adaptation arises from standing genetic variation, it has been predicted to slow down the progress towards speciation under parallel selection, and facilitate progress toward speciation under divergent selection (Thompson et al., 2019). Because the level of genetic variation differs between watercourses, the repeated occurrence of different morphs could be viewed as convergent evolution between watercourses, but parallel among habitats in each of the watercourses (Arendt and Reznick, 2008). Since parallel evolution from standing genetic variation is likely only when different populations adapt to very similar environments (Thompson et al., 2019), a certain degree of non-parallelism could be expected to be common, as ecological conditions may vary between lakes.

Here, I have described the signals of selection in whitefish population across the three watercourses, and how reproductive isolation may arise in sympatry. Thus, these results are in line with the theory of ecological speciation and show how selection has shaped the European whitefish morphs towards stronger phenotype-environment correlation and more diversified populations.

## 5 Conclusions

This thesis highlights the complexities of evaluating the role of random genetic drift, natural selection, and phenotypic and genetic variation, in the process of population differentiation. The results presented herein show how diversifying selection is driving the adaptive divergence, and how

reproductive barriers are forming between populations, eventually leading to reduced gene flow between whitefish morphs within a speciation continuum.

1. Each of the main watercourses seems to form their own entity, where whitefish within watercourses have approximately similar standing genetic variation, suggesting that each of the watercourses was colonized at single events in evolutionary time. Although the morphs were recognised in all three watercourses, there were also lake and watercourse specific differences between whitefish morphs (**Paper I, II, III**). Lakes in the Pasvik watercourse were characterized with the most diverged morphs and the smallest interpopulation coancestry within lakes, whereas in Alta watercourse the divergence was more subtle (**Paper I, II**). Nevertheless, genetic divergence between sympatric morphs was positively associated with phenotypic divergence in all of the three contemporary watercourses.

2. Natural selection has shaped the parallel ecological speciation of whitefish morphs and strengthened the phenotype-environment correlation of European whitefish throughout the lakes in northern Fennoscandia. Whitefish populations in Tana and Alta watercourses, likely due to colonization history and repeated founder events, have been subjected to more genetic drift than populations in oldest Pasvik watercourse (**Paper I**).

3. Allochrony has reinforced the ecological based divergence by building up reproductive isolation and restricting the amount of gene flow between sympatric populations. It has likely been an important factor creating barriers between populations at an early stage of the diverging process (**Paper III**).

## 6 Perspectives

Because phenotypic variation among lineages, genetic mechanisms behind phenotypic alteration, and environmental differences are important components in speciation, all these components should be combined to obtain a more thorough understanding of both phenotypic and genetic divergence in natural populations. Although some adaptive traits have already been detected in European whitefish, high quality phenotypic data combining not just geometric morphometrics, but also physiological traits could assist in detecting more key traits driving the adaptation, and to obtain deeper understanding of the adaptive processes.

High density genetic data would help to investigate the genomic mechanisms of speciation, and coupled with phenotypic data, help to understand the genetic basis of adaptive phenotypes. In addition, little is known about the possible intrinsic postzygotic isolation between morphs. In order to

address this issue, genomic studies of both “pure” and hybrid morphs are needed, as well as comparison between the younger and the older watercourses.

With this more detailed data, comparison of several speciation events experiencing similar environmental pressures would allow us to assess the repeatability of evolution through the identification of similar adaptive characteristics and assess if the ecologically driven speciation across the three watercourses stems from convergent evolution.

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## ORIGINAL RESEARCH

# Diversifying selection drives parallel evolution of gill raker number and body size along the speciation continuum of European whitefish

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## Abstract

Adaptive radiation is the evolution of ecological and phenotypical diversity. It arises via ecological opportunity that promotes the exploration of underutilized or novel niches mediating specialization and reproductive isolation. The assumed precondition for rapid local adaptation is diversifying natural selection, but random genetic drift could also be a major driver of this process. We used 27 populations of European whitefish (*Coregonus lavaretus*) from nine lakes distributed in three neighboring sub-arctic watercourses in northern Fennoscandia as a model to test the importance of random drift versus diversifying natural selection for parallel evolution of adaptive phenotypic traits. We contrasted variation for two key adaptive phenotypic traits correlated with resource utilization of polymorphic fish; the number of gill rakers and the total length of fish, with the posterior distribution of neutral genetic differentiation from 13 microsatellite loci, to test whether the observed phenotypic divergence could be achieved by random genetic drift alone. Our results show that both traits have been under diversifying selection and that the evolution of these morphs has been driven by isolation through habitat adaptations. We conclude that diversifying selection acting on gill raker number and body size has played a significant role in the ongoing adaptive radiation of European whitefish morphs in this region.

## KEYWORDS

adaptation, *Coregonus lavaretus*, drift, gill rakers, phenotype-environment correlation, total length

## 1 | INTRODUCTION

Adaptive radiation is a process where a lineage diversifies into new lineages adapted to divergent environments, which results in phenotype-environment associations, niche specialization, and genetic divergence (Gavrilets & Vose, 2005). Natural selection acts as a driving force for rapid local adaptation (Barrett & Schluter, 2008), whereas random

genetic drift, mutations, and gene flow may play a role in changing gene frequencies in populations. Notwithstanding, nonadaptive processes, such as genetic linkage and pleiotropy of traits, may also play important roles in the phenotypic and genotypic differentiation involved in adaptive radiations (Schluter, 2000). The genetic architecture of traits, together with the adaptive genetic variation, upon which various selection pressures are exerted, will set the frame for the response

to natural selection (Nosil, Funk, & Ortiz-Barrientos, 2009). However, divergence of populations, and eventually speciation, can also occur via random genetic drift (Lee, Shaner, Lin, & Lin, 2016; Uyeda, Arnold, Hohenlohe, & Mead, 2009). Because of this complexity, the mechanisms by which evolution modulates phenotypic and genotypic frequencies in the divergence process are not well understood. In order to detect selection and rule out other causes as an explanation for the differentiation of phenotypic traits, it is necessary to compare the observed differentiation with neutral differentiation expected under random genetic drift. Although this has been attempted by contrasting neutral genetic ( $F_{ST}$ ) and quantitative trait ( $Q_{ST}$ ) differentiation (Brommer, 2011; Østbye, Næsje, Bernatchez, Sandlund, & Hindar, 2005; Ozerov et al., 2015; Whitlock, 2008), assessing the interplay between environmental and genetic causes of differentiation has been problematic as  $F_{ST}/Q_{ST}$  comparison does not allow for detection of interactions between phenotypes, genotypes, and the environment (Pujol, Wilson, Ross, & Pannell, 2008). Therefore, it is often challenging to disentangle whether the observed differentiation in phenotypic traits is a response to natural selection or simply just a plastic response to environmental differences, especially when the number of populations is small and they are subject to strong random genetic drift (Brommer, 2011; Leinonen, McCairns, O'Hara, & Merilä, 2013; Ovaskainen, Karhunen, Zheng, Arias, & Merilä, 2011; Pujol et al., 2008). However, recent efforts in coupling quantitative and population genetic theory have created realistic models (Ovaskainen et al., 2011) and tools (R package "driftsel," Karhunen, Merilä, Leinonen, Cano, & Ovaskainen, 2013), for this exercise. Using the Bayesian methods implemented in "driftsel," it is now possible to contrast and statistically test differentiation of phenotypic traits under scenarios of random genetic drift and diversifying selection, and thereby compare possible similarities among phenotypes and environments (e.g., habitats) even with small number of populations or when  $Q_{ST}$  equals  $F_{ST}$  (Ovaskainen et al., 2011).

The numerous postglacial lakes harboring polymorphic fish populations in Fennoscandia are relatively young (<10 kyr) and represent discrete and isolated environments, making them outstanding "natural laboratories" for studying processes that initiate and maintain niche adaptation and population divergence. European whitefish (*Coregonus lavaretus* [L.]) is a highly abundant fish species in these lakes and has diverged into distinct morphs adapted to the three principal lake habitats (littoral, pelagic, and profundal; Harrod, Mallela, & Kahilainen, 2010; Kahilainen & Østbye, 2006; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2010). The morphs are discriminated based on the head morphology and the number of gill rakers (Amundsen, Bøhn, & Våga, 2004; Kahilainen & Østbye, 2006; Siwertsson et al., 2010). In addition, the morphs differ in body size, where the large-bodied individuals are found in the most profitable foraging habitat, the littoral zone, whereas smaller individuals are found in the pelagic and profundal zones (Bøhn & Amundsen, 2004; Kahilainen, Alajärvi, & Lehtonen, 2005; Kahilainen, Lehtonen, & Könönen, 2003). The number of gill rakers is a heritable and ecologically important trait (Bernatchez, 2004; Svärdson, 1952, 1979), associated with diet preference (Østbye et al., 2006; Siwertsson, Knudsen, Adams, Præbel, & Amundsen, 2013). The

whitefish morphs are named according to their body size and the number of gill rakers (Kahilainen & Østbye, 2006) as follows: large sparsely rakered (LSR) whitefish with intermediate number of gill rakers, densely rakered (DR) whitefish with the highest number of gill rakers, and small sparsely rakered (SSR) whitefish with the lowest number of gill rakers. LSR whitefish mainly feeds on benthic macroinvertebrates in the littoral habitat, DR whitefish is a zooplanktivorous specialist that resides in the pelagic habitat, and SSR is a specialized benthivore consuming profundal benthic invertebrates (Harrod et al., 2010).

Previous studies have suggested that the variation in the degree of divergence among the morphs throughout northern Fennoscandia represents a speciation continuum within watercourses and at a broader landscape level (Østbye, Næsje, et al., 2005; Østbye et al., 2006; Siwertsson et al., 2010). The ubiquitous LSR whitefish has been regarded as the ancestral phenotype from which the other morphs have evolved (Østbye, Bernatchez, Næsje, Himberg, & Hindar, 2005), as this morph is present in all lakes and the only morph found in allopatriy. The most diversified systems in this region are found close to the main stem in the easternmost Pasvik watercourse (Siwertsson et al., 2010), where most lakes harbor all three whitefish morphs (DR, LSR, and SSR; Harrod et al., 2010; Kahilainen & Østbye, 2006; Præbel, Knudsen, et al., 2013). The same pattern of the main stem lakes being the most diversified also holds true for the more western watercourses, despite that the radiations are less developed. In the Tana and Alta watercourses, all three morphs are phenotypically recognized, but the SSR morph appears to be genetically less diverged from the LSR morph compared to the radiation in Pasvik (Præbel, Knudsen, et al., 2013; Siwertsson et al., 2010; Siwertsson, Knudsen, Præbel, et al., 2013). Intraspecific and interspecific genetic diversity of the whitefish morphs also decreases from the Pasvik to the Alta watercourse, so that the allelic richness in the Tana and Alta watercourses is just a subset of the allelic richness found in the Pasvik watercourse (Østbye et al., 2006). The reduced allelic richness in the Tana and Alta watercourses has been hypothesized to be related to the postglacial colonization route from east to west by a single clade of whitefish (Østbye, Bernatchez, et al., 2005). The postglacial colonization likely followed the retreating ice sheet edge from east to west about 10,000 years B.P. (Andersen & Borns, 1994; Kujansuu, Eriksson, & Grönlund, 1998; Mangerud et al., 2004; Sollid et al., 1973; Svendsen et al., 2004). As a consequence, the whitefish populations in the Pasvik watercourse are expectedly 5,000 years older compared to the whitefish populations in the Alta watercourse (Præbel, Knudsen, et al., 2013).

There is a considerable amount of ecological studies that suggest natural selection as a main cause behind divergent whitefish populations (e.g., Amundsen et al., 2004; Lu & Bernatchez, 1999; Siwertsson et al., 2010), but only a few studies have attempted to test whether phenotypic traits, gill raker number and body size have an adaptive role in the divergence process in whitefish (Østbye, Næsje, et al., 2005; Præbel, Knudsen, et al., 2013; Rogers, Gagnon, & Bernatchez, 2002; Vonlanthen et al., 2009). Divergence in gill raker and body size traits is commonly detected in a range of different postglacial fish species along speciation continuums, suggesting their key importance toward increasing specialization into pelagic or

benthic niches (Hendry et al. 2009). Previous phenotypic-genotypic variation comparisons of gill raker counts in pelagic and benthic lake whitefish (*Coregonus clupeaformis*) and European whitefish have revealed deviation from neutral expectation, suggesting that the number of gill rakers has evolved as a product of natural selection (Østbye, Næsje, et al., 2005; Præbel, Knudsen, et al., 2013; Rogers et al., 2002). However, these previous studies have focused on single or a few neighboring lakes, whereas landscape level approaches are missing. Range expansion of species to new areas generally leads to reduced allelic richness and heterozygosity (Besold, Schmitt, Tammaru, & Cassel-Lundhagen, 2008; White, Perkins, Heckel, & Searle, 2013). These repeated founder events build up genetic differentiation through a spatial analog of genetic drift (Slatkin & Excoffier, 2012). In the case of northern Fennoscandian whitefish, the range expansion from the oldest Pasvik watercourse populations toward the progressively younger Tana and Alta watercourse populations likely conserve the footprints of the colonization history as, for example, manifested in a diminishing amount of genetic variation. However, whether evolution of similar adaptive phenotypes in different lakes and watercourses with repeated founder events are driven by diversifying selection or originate from random genetic drift, remains to be tested.

This study assesses the different stages of divergence along the speciation continuum for all three whitefish morphs throughout the wide northern Fennoscandian landscape. Our objectives were to investigate i) whether diversifying selection or the repeated events of genetic drift from the postglacial recolonization has shaped phenotypic traits of whitefish into three different adaptive modes in the three lake habitats, and ii) whether the habitat is more important for the observed patterns of natural selection than lakes or watercourses, thus reflecting the action of parallel evolution. If random genetic drift is the main driver of the phenotypic divergence, we expect to observe

random genotypic and phenotypic clustering of morphs within lakes and watercourses.

## 2 | MATERIALS AND METHODS

We collected whitefish in nine lakes from three subarctic watercourses: Suohpatjavri, Stuorajavri, and Vuolgamasjavri from Alta watercourse, Iddjavri, Vuoddasjavri, and Pulmankijärvi from Tana watercourse, and Inarijärvi, Skrukkebukta, and Langfjordvatn from Pasvik watercourse (Figure 1). We chose large (2–32 km<sup>2</sup>) and deep (max 25–53 m) oligotrophic lakes where all the three principal habitats (littoral, pelagic, and profundal) were present. Fish were sampled from all habitats using benthic and pelagic gill net series (mesh sizes 10–60 mm). The fish were removed from gill nets and visually classified to morph according to appearance, head, and body form, and by their gill raker morphology as described by Kahilainen and Østbye (2006). The total length (accuracy 1 mm) was measured; fin clip or a piece of gill filament was taken for genetic analyses, followed by visual inspection of gonads for sex determination and assessment of sexual maturity. The number of gill rakers was counted under a microscope on the first left branchial arch to verify the right morph assignment. Individuals with undefined morph record or morphs caught in a non-native habitat were excluded. Without physical boundaries between different lake habitats, the morphs are not restricted only to their native habitat. However, analyses of stomach contents have uncovered different diet preferences and low niche overlap between the three distinct whitefish morphs (Harrod et al., 2010; Kahilainen & Østbye, 2006; Østbye et al., 2006). These criteria lead to a dataset with a total number of 999 individuals, from which we had the following information: morph classification, sex, maturity, number of gill rakers, total length, and data from 21 microsatellite loci.



**FIGURE 1** Map of northern Europe and close up of the northern Fennoscandian location of the study sites. All three watercourses are well separated and drain to different fjords in the Arctic Ocean. Open dots indicate study lakes in the Pasvik watercourse, gray dots the Tana watercourse, and black dots the Alta watercourse. For Inarijärvi, the dot is located to the specific sampling site within the lake



Stable isotope analyses in trimorphic lakes in the Pasvik watercourse have showed differences in resource use between morphs (Harrod et al., 2010; Præbel, Knudsen, et al., 2013). Despite the less prominent divergence between LSR and SSR whitefish in the Alta watercourse, the stable isotope ratios of carbon and nitrogen have also there revealed two ecological clusters, the profundal and the littoral, within the benthic whitefish, demonstrating that the capture habitat of the benthic fish is a good indicator of long-term resource use (Siwertsson, Knudsen, Præbel, et al., 2013). We assume that the same association holds true for the Tana watercourse—logically also—due to the colonization history and its location between the Pasvik and Alta watercourses. In addition to the habitat and dietary segregation, littoral and profundal whitefish in the Alta watercourse differ in head morphology (Siwertsson, Knudsen, Adams, et al., 2013). Further, small but statistically significant genetic differences have also been observed between littoral and profundal whitefish (Siwertsson, Knudsen, Præbel, et al., 2013).

The number of gill rakers in the different morphs represented three partly overlapping unimodal distributions where SSR had gill raker number from 18 to 24, LSR from 22 to 39, and DR from 27 to 41. SSR whitefish has shorter gill rakers, robust head, and larger eyes compared to LSR whitefish, whereas DR has long gill rakers and pointed head shape (Harrod et al., 2010; Kahilainen & Østbye, 2006). In order to study the populations at different positions along the speciation continuum, whitefish caught in the profundal habitat with gill raker counts 28 or lower, were coded as SSR whitefish in Tana and Alta watercourses (but see also Siwertsson et al., 2010; Siwertsson, Knudsen, Adams, et al., 2013; Siwertsson, Knudsen, Præbel, et al., 2013). As the SSR whitefish in the Tana and Alta watercourses are less diverged from the LSR whitefish than in the Pasvik watercourse, the upper limit for the number of gill rakers was set at a higher number than what the SSR whitefish in Pasvik watercourse usually have, and thus overlapped more with LSR whitefish.

## 2.1 | Phenotypic traits

We assessed the divergence among morphs with regard to two common phenotypic traits of postglacial fish radiations (Hendry, 2009; Schluter, 2000): body size (the total length of fish) and the number of gill rakers. The number of gill rakers is known to be a highly heritable trait (Svärdson, 1952, 1979), which also correlates with dietary niche and obviously shows a trait utility in terms of foraging (Kahilainen et al., 2011; Østbye et al., 2006). The correlation between parents and progeny with regard to the number of gill rakers is strong, as the heritability estimate ( $h^2$ ) for the trait is high (0.79; Bernatchez, 2004; Svärdson, 1952, 1979). Heritability estimates for body length in salmonid fish vary from 0.08 to 0.42 (Gjerde & Gjedrem, 1984; Gjerde & Schaeffer, 1989; Gunnes & Gjedrem, 1978; Refstie & Steine, 1978; Standal & Gjerde, 1987). As a phenotypic trait, the body length not only represents the size of the fish but also often strongly correlates with weight, condition, age, maturity, and sex. The smaller heritability estimates for body length also mean that the effect of additive

**TABLE 1** Spatial and morphometric information of the study lakes, that is, lake area, maximum depth, altitude, number of fish species present, and location of the lake

Lake	Area (km <sup>2</sup> )	Max depth (m)	Altitude (m a.s.l.)	No of fish species	Latitude (°N)	Longitude (°E)	N	Males	Females	N.A.	Mature (%)
Suohpatjavri	2.0	25	325	5	68° 56'	23° 05'	82	41	41		62
Stuorajavri	23.7	30	374	6	69° 06'	22° 49'	111	61	50		34
Vuolgamasjavri	2.8	30	301	6	69° 07'	23° 20'	114	52	62		45
Iddjajavri	6.4	30	275	5	69° 37'	25° 16'	141	74	50	17	44
Vuoddasjavri	2.9	32	334	5	69° 21'	24° 00'	142	91	51		61
Pulmankijävi	12.0	36	12	9	70° 00'	28° 01'	123	50	72	1	59
Inarjävri	32.0 <sup>a</sup>	40 <sup>a</sup>	118	13	69° 02'	27° 52'	71	26	30	15	35
Skrukkebukta	6.6	37	21	8	69° 33'	30° 06'	87	44	41	2	51
Langfordvatn	2.8	53	7	6	69° 33'	29° 57'	128	63	63	2	91

N, total amount of fish used in analyses; males/females, number of males/females; N.A., individuals with unknown sex; mature, proportion of mature fish on each lake.  
<sup>a</sup>In Inarjävri, sampling was confined to a single 32 km<sup>2</sup> bay (Nanguvuono).

genetic variation for this trait is smaller and that environmental factors may have more effect on the phenotypic difference in total length of fish than in the number of gill rakers. Although the number of mature and immature individuals was fairly equal in our dataset (Table 1), we added maturity and sex as fixed effects in our model to account for the age of the fish and possible effects of sexual dimorphism on the phenotypic traits. Both fixed effects were treated as binary traits. The dataset included 37 individuals with unknown sex and 19 individuals with unknown maturity records (Table 1). As sex was not associated with the total length of fish or the number of gill rakers, missing data for these traits were considered not to affect the further analyses. The overall proportion of males and females in the data was 50% and 46%, respectively, while 55% of all individuals were mature (Table 1). The proportion of males in the study lakes ranged from 42% to 64%, whereas the proportion of mature fish in the samples varied from 35% to 91% among lakes (Table 1). The number of gill rakers and the total length of fish among the three morphs in all the lakes in the three watercourses were compared using analyses of variance, and multiple pairwise comparisons were performed with Tukey's HSD test in the R statistical computing programme (R Core Team, 2017).

## 2.2 | Microsatellite DNA/genotyping

Genomic DNA was extracted using E-Z96 Tissue DNA Kit (OMEGA Bio-tek) following the manufacturer's instructions. A total of 21 microsatellite loci (Table S1) were amplified in four polymerase chain reaction (PCR) multiplexes using forward-labeled primers according to the protocol of Præbel, Westgaard, et al. (2013). The PCR products were separated on an ABI 3130 XL Automated Genetic Analyser (Applied Biosystems) using GENESCAN LIZ-500 (Applied Biosystems) as an internal size standard. The binning and scoring were 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 the absence of contamination. The repeatability and consistency of genotypes were 100%, and contamination was absent. The genotypes were screened for abnormalities in the software MICRO-CHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004), using 1,000 bootstraps to generate the expected homozygote and heterozygote allele size difference frequencies. The microsatellite data were tested with LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008) to obtain neutral marker data. The neutral marker data contained 13 microsatellite loci (bolded in Table S1). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested per locus over all populations using exact tests (Guo & Thompson, 1992) as implemented in GenePop 4.0 (Rousset, 2007). The pairwise comparisons were corrected for multiple comparisons using sequential Bonferroni corrections (BFC) following Rice (1989). The number of alleles at each microsatellite locus ranged from 6 to 36 across all lakes and morphs (Table S1). Deviations from HWE were indicated in 13 of 351 tests (3.7%) after sequential BFC, which are less than expected by chance

(5%). None of the loci comparisons ( $n = 2,106$ ) were significant for LD after BFC.

## 2.3 | Model for genetic differentiation

We compared neutral genetic differentiation and observed quantitative genetic differentiation in order to differentiate random genetic drift from selection. Under random genetic drift, the vector of population means  $a^p$  has the multivariate normal distribution

$$a^p \sim N(\mu^A, 2G^A \otimes \theta^p)$$

where  $\mu^A$  is the common ancestral mean for all populations,  $G^A$  is the ancestral variance–covariance matrix summarizing the variances and covariance of traits,  $\otimes$  is a Kronecker product, an operator resulting block matrix, and  $\theta^p$  is the population-level coancestry matrix. The analyses were performed with RAFM (Karhunen & Ovaskainen, 2012) and Driftsel R packages (Karhunen et al., 2013). Driftsel requires two types of data, genotypic data from neutral molecular markers and quantitative data from phenotypes. The genotypic data from the 13 neutral microsatellite loci were analyzed with R package RAMF in order to obtain posterior distribution of the neutral genetic differentiation  $\theta^p$  (coancestry coefficients). The coancestry coefficient is the summarization of the expected level of genetic similarity; in other words, it evaluates how much the individuals are expected to resemble each other. Estimation was calculated with an admixture F-model (Karhunen & Ovaskainen, 2012) using 200,000 iterations, 50,000 burning iterations, and a thinning interval of 100. The posterior distribution from the coancestry matrix  $\theta^p$  was then used as a prior for Driftsel to estimate the posterior distributions of other parameters and to refine the estimate of  $\theta^p$ . The function MH (a Metropolis–Hastings algorithm for quantitative genetics) in Driftsel was executed using 80,000 iterations, 40,000 burning iterations, and thinning interval of 10. Convergence of analysis was qualitatively evaluated based on visual inspection of three parallel runs. We analyzed all the three watercourses, altogether nine lakes, in one overall round, and then subsequently divided the data into separate watercourses to investigate the effect of watercourse. As Driftsel does not specify clearly which traits selection is acting on, we ran each trait separately in addition to an overall round with both traits.

The effect of physical proximity and habitat types to population structure was assessed by comparing the levels of coancestry between lakes and between habitats within each watercourse. We used the population coancestry coefficient  $\theta^p$  to calculate the average coancestry within habitats (between lakes) and within lakes (between habitats) to investigate whether the population structure was more influenced by habitat or by lake.

In addition, we performed the formal  $S$  and  $H$  tests (Karhunen, Ovaskainen, Herczeg, & Merilä, 2014; Ovaskainen et al., 2011). Both of these tests use posterior distributions calculated with Driftsel as a prior (MH function). The  $S$  test evaluates how far the population

**TABLE 2** Summary table of nine study lakes indicating watercourse, lake, whitefish morph code, sample size (N), mean number and range of gill rakers as well as mean total length (cm) for each population

Watercourse	Lake	Morph	Code	N	Mean gill rakers ± SD (min–max)	Mean length ± SD (min–max)
Alta	Suohpatjavri	DR	SuD	33	40.7 ± 2.6 (37–47)	24.0 ± 1.1 (21.8–25.5)
		LSR	SuL	34	28.2 ± 2.1 (24–32)	24.4 ± 6.0 (16.5–35.5)
		SSR	SuS	15	23.7 ± 3.3 (18–28)	27.1 ± 4.0 (20.2–36.6)
	Stuorajavri	DR	StD	44	34.8 ± 2.1 (31–40)	22.0 ± 2.8 (13.1–29.5)
		LSR	StL	39	24.1 ± 1.9 (21–28)	21.0 ± 5.3 (14.0–33.7)
		SSR	StS	28	23.2 ± 2.2 (19–26)	19.7 ± 3.7 (14.0–30.1)
	Vuolgamasjavri	DR	VgD	22	37.8 ± 2.3 (33–41)	22.3 ± 2.6 (17.5–27.7)
		LSR	VgL	50	25.3 ± 1.7 (20–30)	23.5 ± 5.4 (11.5–35.1)
		SSR	VgS	42	23.3 ± 2.5 (19–28)	22.0 ± 5.4 (15.5–35.2)
Tana	Iddjajavri	DR	IdD	62	34.0 ± 2.1 (29–39)	17.2 ± 2.1 (12.6–25.0)
		LSR	IdL	56	22.1 ± 3.4 (15–34)	23.9 ± 7.5 (9.8–40.7)
		SSR	IdS	23	22.1 ± 2.0 (19–26)	17.9 ± 4.5 (11.2–28.0)
	Vuoddasjavri	DR	VdD	51	38.0 ± 2.5 (28–42)	13.0 ± 2.1 (9.3–22.1)
		LSR	VdL	48	24.2 ± 2.8 (18–31)	19.4 ± 3.7 (10.5–27.2)
		SSR	VdS	43	22.0 ± 2.1 (17–27)	20.8 ± 3.9 (10.2–33.3)
	Pulmankijärvi	DR	PuD	55	40.0 ± 2.2 (36–45)	23.5 ± 1.4 (17.1–26.2)
		LSR	PuL	31	24.2 ± 1.8 (22–28)	23.5 ± 7.7 (13.5–57.0)
		SSR	PuS	37	23.9 ± 1.6 (20–28)	28.7 ± 2.5 (20.8–32.7)
Pasvik	Inarijärvi	DR	InD	26	35.1 ± 2.6 (29–39)	11.5 ± 3.0 (6.1–17.4)
		LSR	InL	22	21.7 ± 1.4 (19–25)	26.9 ± 6.6 (18.3–46.1)
		SSR	InS	23	18.0 ± 1.4 (16–21)	21.5 ± 3.2 (13.9–25.3)
	Skrukkebukta	DR	SbD	16	33.1 ± 3.1 (29–40)	13.7 ± 3.2 (10.0–20.5)
		LSR	SbL	32	24.9 ± 2.5 (21–31)	19.9 ± 5.9 (7.7–30.4)
		SSR	SbS	39	20.0 ± 1.8 (16–23)	16.3 ± 1.9 (12.0–21.6)
	Langfjordvatn	DR	LfD	20	35.2 ± 3.0 (27–40)	11.9 ± 0.8 (11.3–15.0)
		LSR	LfL	61	27.3 ± 5.3 (20–39)	16.3 ± 4.1 (10.9–30.0)
		SSR	LfS	47	22.6 ± 2.4 (17–29)	20.2 ± 2.9 (15.5–29.5)

Abbreviations of morphs are DR, densely rakered whitefish; LSR, large sparsely rakered whitefish; SSR, small sparsely rakered whitefish. Code is a combination of lake and morph name.

means have diverged (or drifted) from the ancestral mean. It detects the signals of selection by comparing the posterior distributions of the population effects, variance–covariance matrix of the traits and the  $\theta^P$  from neutral marker data. The  $H$  test measures whether population means correlate with the environmental data more than would be expected on the basis of shared evolutionary history. It includes environmental data to the neutrality test and assesses the similarities of populations found in similar habitats. The  $S$  and  $H$  tests values range from zero to one. Values close to one and zero from the  $S$  test imply diversifying and stabilizing selection, respectively, whereas values close to 0.5 imply perfect match with neutrality.  $S$  test is known to be conservative, where a  $S$  value of 0.95 refers to diversifying selection at 95% credibility level (Karhunen et al., 2014). A large  $H$  test value suggests that populations are more adapted to their environment than would be expected based on their shared phylogenetic history (Karhunen et al., 2014).

### 3 | RESULTS

#### 3.1 | Phenotypic analysis

The number of gill rakers ranged from 15 to 47 with an overall mean of  $28 \pm 7.1$  ( $\pm$ SD; Table 2). The mean number of gill rakers was significantly different ( $p < .001$ ) between different morphs within all lakes in the Pasvik watercourse, where DR whitefish had the highest ( $34.6 \pm 3.0$ ), LSR whitefish intermediate ( $25.6 \pm 4.7$ ), and SSR whitefish the lowest ( $20.7 \pm 2.7$ ) number of gill rakers (Table 3). Also in Tana and Alta, the mean number of gill rakers was higher for DR whitefish ( $p < .001$ ; Tana:  $37.2 \pm 3.4$ , Alta:  $37.4 \pm 3.5$ ) compared to LSR (Tana:  $23.4 \pm 3.1$ , Alta:  $25.7 \pm 2.5$ ) and SSR whitefish (Tana:  $22.7 \pm 2.1$ , Alta:  $23.4 \pm 2.6$ ). On the contrary, when comparing the LSR and SSR whitefish, we found significant differences for number of gill rakers only in Suohpatjavri in Alta ( $p < .001$ ) and Vuoddasjavri

**TABLE 3** Summary trait table of the three morphs in each watercourse indicating mean number of gill rakers and total length (cm) of fish  $\pm$  standard deviation (*SD*). Statistical significance of traits among subpopulations within lakes on different watercourses is indicated with asterisks

Watercourse	Morph	Mean number of gill rakers $\pm$ <i>SD</i>	DR	LSR	Mean length $\pm$ <i>SD</i>	DR	LSR
Alta	DR	37.4 $\pm$ 3.5			22.8 $\pm$ 2.5		
	LSR	25.7 $\pm$ 2.5	***		22.9 $\pm$ 5.7	—	
	SSR	23.4 $\pm$ 2.6	***	***/—	22.2 $\pm$ 4.8	—	—
Tana	DR	37.2 $\pm$ 3.4			18.0 $\pm$ 4.7		
	LSR	23.4 $\pm$ 3.1	***		22.2 $\pm$ 6.7	***/—	
	SSR	22.7 $\pm$ 2.1	***	*/—	22.9 $\pm$ 5.7	***/—	***/—
Pasvik	DR	34.6 $\pm$ 3.0			12.2 $\pm$ 2.7		
	LSR	25.6 $\pm$ 4.7	***		19.4 $\pm$ 6.5	***	
	SSR	20.7 $\pm$ 2.7	***	***	19.1 $\pm$ 3.4	***/—	***/**

\*\*\* $p < .001$ , \*\* $p < .01$ , \* $p < .05$ , — N.S.

in Tana ( $p < .001$ ), although in Vuolgamajavri, the differences in number of gill rakers were close to be statistically significant ( $p = .055$ ; Table 2). The mean total length of DR whitefish was generally smaller ( $18.3 \pm 5.2$  cm [*SD*]) than the mean total length of LSR ( $21.6 \pm 6.5$  cm) and SSR ( $21.3 \pm 5.0$  cm) whitefish ( $p < .001$ ), but the total length of fish varied between lakes and watercourses (Tables 2 and 3). In the Pasvik watercourse, DR whitefish were smaller ( $12.2 \pm 2.7$  cm) than LSR ( $19.4 \pm 6.5$  cm) whitefish, but there were no statistically significant differences between DR and SSR whitefish in Skrukkebukta. Also in Tana, the DR whitefish ( $18 \pm 4.7$  cm) were smaller in size than the other morphs (LSR:  $22.2 \pm 6.7$  cm, SSR:  $22.9 \pm 5.7$  cm), with a few exceptions: There were no statistically significant differences between DR and LSR whitefish in Pulmankijärvi, between DR and SSR whitefish in Iddjavri, and between LSR and SSR whitefish in Vuoddasjavri (Table 3). In Alta, the mean lengths of all three morphs were greater than in Pasvik, but there were no significant differences in the mean total length among the morphs (DR:  $22.8 \pm 2.5$  cm; LSR:  $22.9 \pm 5.7$  cm; SSR:  $22.2 \pm 4.8$  cm) in any of the three lakes.

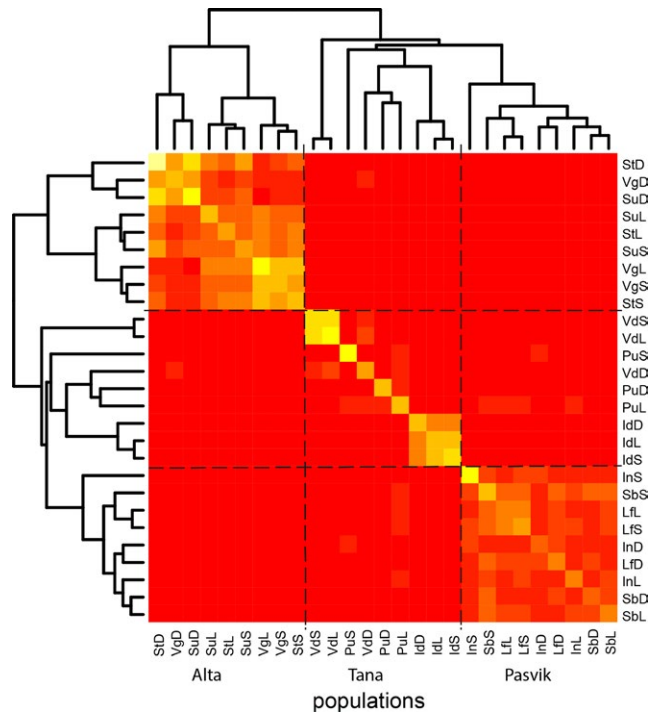
### 3.2 | Population-to-population coancestry matrix

The population-level coancestry coefficient matrix  $\theta^P$  illustrates the relatedness between populations (Table S2). Mean estimates of diagonal elements in the coancestry matrix ( $\theta_{ii}^P$ ) were greater in Alta and Tana than in Pasvik, suggesting that the whitefish morphs in Alta and Tana have been subjected to more genetic drift than the whitefish morphs in the Pasvik watercourse. The off-diagonal elements of the coancestry coefficients ( $\theta_{ij}^P$ ) represent the interpopulation coancestry and the gene flow between populations. The level of relatedness between morphs was largest in Alta, where especially DR whitefish in different lakes clustered together and displayed high interpopulation coancestry among them, whereas relatedness between DR and the benthic morphs was smaller (Figure 2). When comparing the level of coancestry between lakes and between habitats, the differences between the three watercourses were prominent. In Alta, the average

coancestry within morphs across lakes was larger than the average coancestry within lakes among morphs (Table S2). In other words, relatedness between morphs across lakes was larger than relatedness of different morphs within same lakes. Thus, the population structure was more dependent on the effect of habitat than the effect of the lake. Tana was characterized by low levels of gene flow between lakes, although the coancestry coefficient revealed low levels of relatedness between Tana, Alta, and Pasvik watercourses. However, all except one of these off-diagonal terms of relatedness between different watercourses were  $\leq 0.01$  (between PuL and SbS: 0.02), which can refer to numerical noise from the MCMC calculation (Table S2). The most diverged morphs with the smallest interpopulation coancestry within lakes were in Pasvik. Although the morphs were related also across the lakes, the average relatedness within lakes was slightly greater than the average relatedness among morphs in different lakes.

### 3.3 | Observed divergence versus expected divergence under drift

Neutral divergence in phenotypic traits was assessed using the ancestral mean value and the expected divergence under random genetic drift and then compared to observed divergence on phenotypic traits (Figure 3). Signals of selection were detected for most populations and were most pronounced for the DR and the SSR whitefish (Figure 3a–d). Because the trait visualization in Driftsel is a summary statistic that combines the two traits, we also examined the estimates of population means  $\pm$  from real data (population effect) against the ancestral mean and neutral divergence for both traits separately to gain a more detailed impression of these two traits (Figure 4). When comparing the population effect for gill raker number, the DR whitefish in all the watercourses, as well as SSR whitefish in Pasvik watercourse, showed substantial differences from the ancestral mean and drift-based estimates (Figure 4a). Thus, diversifying selection appears to have more effect than random genetic drift on the number of gill rakers in these two morphs.



**FIGURE 2** Heat map illustration of the coancestry matrix  $\theta^P$ . Watercourses are separated with hatched lines. The lighter the color is, the more the populations resemble each other. Diagonal elements of matrix  $\theta_{ii}^P$  represent random genetic drift. Dendrogram on the side illustrates the structure and hierarchical clustering of the genetic matrix. For lake and morph codes, see Table 2

In Tana and Alta, the difference between the observed population effect and the drift-based divergence in the SSR morph was a bit smaller (Figure 4a). Although the SSR morph was further from the ancestral mean in most of lakes in these two watercourses than what would have been expected without selection, the SSR morph in Suohpatjavri, Iddjajarvi, and Pulmankijarvi was still close to the neutral drift-based divergence. When assessing the length of fish, the effect of population was not as clear as with the gill raker number (Figure 4b). The population means were not very far from the ancestral mean and were also close to the estimated drift-based divergence. Also, here the DR whitefish showed evident separation from the ancestral mean, although the length of fish had a smaller population effect for the DR whitefish compared to the other morphs. This was most clear in the Pasvik watercourse, where the population mean for DR whitefish was moved further away from the ancestral mean than expected based on the drift alone, and DR whitefish were smaller in size than the other morphs (Figure 4b). In Alta and Tana watercourses, the populations were (with some exceptions) in general closer to the neutral drift-based estimates. In other words, the divergence in phenotypic traits was not as pronounced in Alta and Tana watercourses as observed in the Pasvik watercourse.

In order to evaluate the effect of selection versus drift on single traits, we estimated  $S$  and  $H$  test values separately for both traits. The  $S$  test values for both traits were  $>0.95$  in all three watercourses,

which confirms that the differences in population means of the traits have been caused by selection as the observed diversification cannot be achieved by random genetic drift alone. The signal of selection was clear when adding habitat information to the statistical test as the  $H$  test values were  $>0.99$ . One exception was the  $H$  test  $-$ value (0.97) of fish length in Alta watercourse. The high values ( $>0.95$ ) observed in the  $H$  test in all watercourses confirmed that there is a strong correlation between the phenotypic traits and the environment.

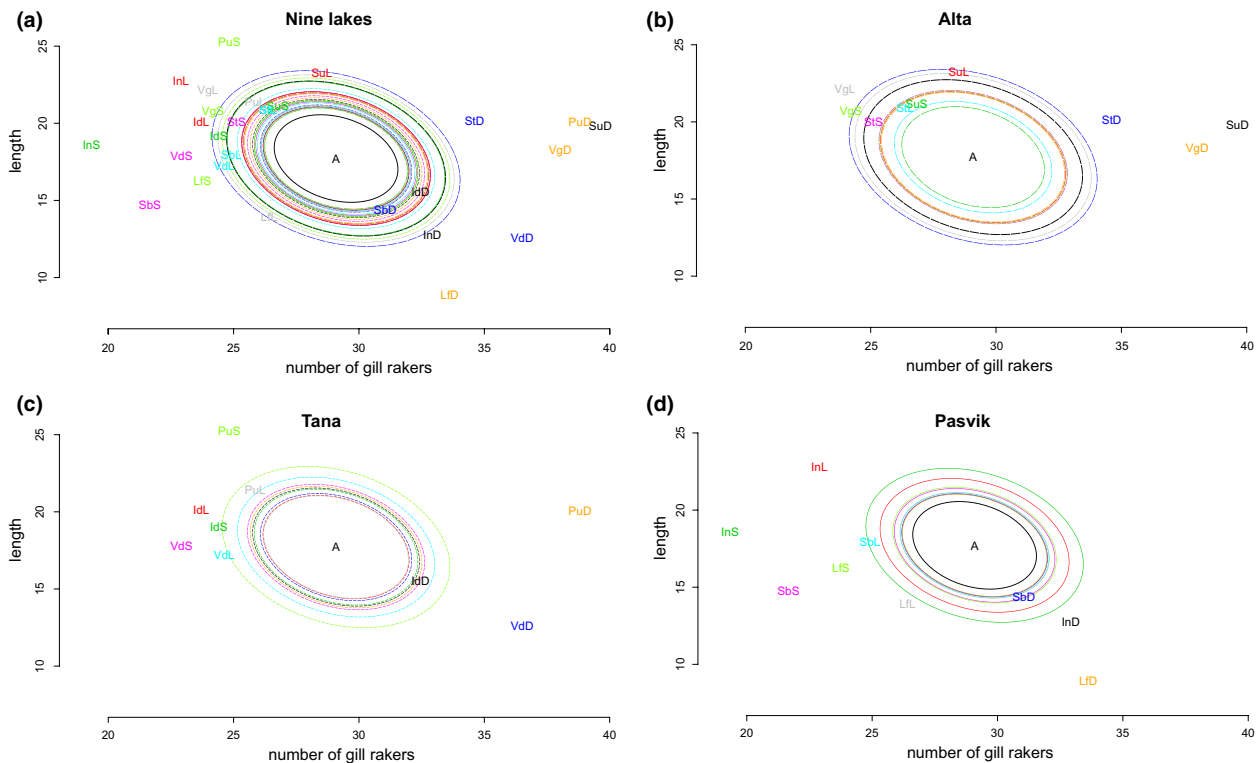
We found a small antagonistic relationship between number of gill rakers and length, suggesting that a higher number of gill rakers tend to be related with smaller size of fish (Figure 4). This relationship was least visible in Alta, where there were no significant differences in mean length between the whitefish morphs (Figure 4). Evaluation of the fixed effects revealed size difference between sexes, where males were slightly smaller (mean  $20.2 \pm 5.6$  cm [SD]) than females (mean  $20.8 \pm 5.9$  cm). This difference was small and statistically significant only for the LSR whitefish in Suohpatjavri and for DR and SSR whitefish in Inarijärvi. Immature fish were also slightly smaller (mean  $18.9 \pm 5.4$  cm) than mature fish (mean  $21.5 \pm 5.9$  cm;  $p < .001$ ).

## 4 | DISCUSSION

In the present study, we tested whether extrinsic factors repeatedly have shaped the phenotypic specializations, number of gill rakers, and fish length, in three sympatric whitefish morphs. Using a landscape-wide study design, we also tested whether the phenotypic specializations were driven by parallel evolutionary processes. The results of our study show that phenotypic differentiation in the three whitefish morphs was a response to diversifying selection, as neutral drift-based divergence was not able to explain the observed pattern. We observed parallel phenotype-environment association especially in number of gill rakers among the whitefish morphs across the lakes of three watercourses.

Our results are in line with the theory of ecological speciation, which predicts that reproductive isolation evolves between populations as a by-product of ecologically based diversifying selection (Nosil, 2012). Diversifying selection mediates the development of adaptive phenotypic traits, such as gill rakers and body size, allowing for more efficient niche utilization (Schluter, 2000). Resource competition and subsequent adaptation to a specific niche are suggested to be driving mechanisms in this divergence process (Pfennig & Pfennig, 2010), but only a few empirical studies have investigated how diversifying selection contributes to the divergence at the early stages of ecological speciation (see Bolnick & Fitzpatrick, 2007; Meyer & Kautt, 2014 for review). This also applies for the most studied examples of ecological speciation, the very diverse cichlids assemblages in tropical lakes (Malinsky et al., 2015; Seehausen, 2006), the pelagic-benthic threespine sticklebacks (*Gasterosteus aculeatus*; Arnegard et al., 2014; McKinnon & Rundle, 2002), and Arctic charr (*Salvelinus alpinus*; Klemetsen, 2010; Recknagel, Hooker, Adams, & Elmer, 2017; Snorrason et al., 1994) in postglacial lakes. The existence of the three



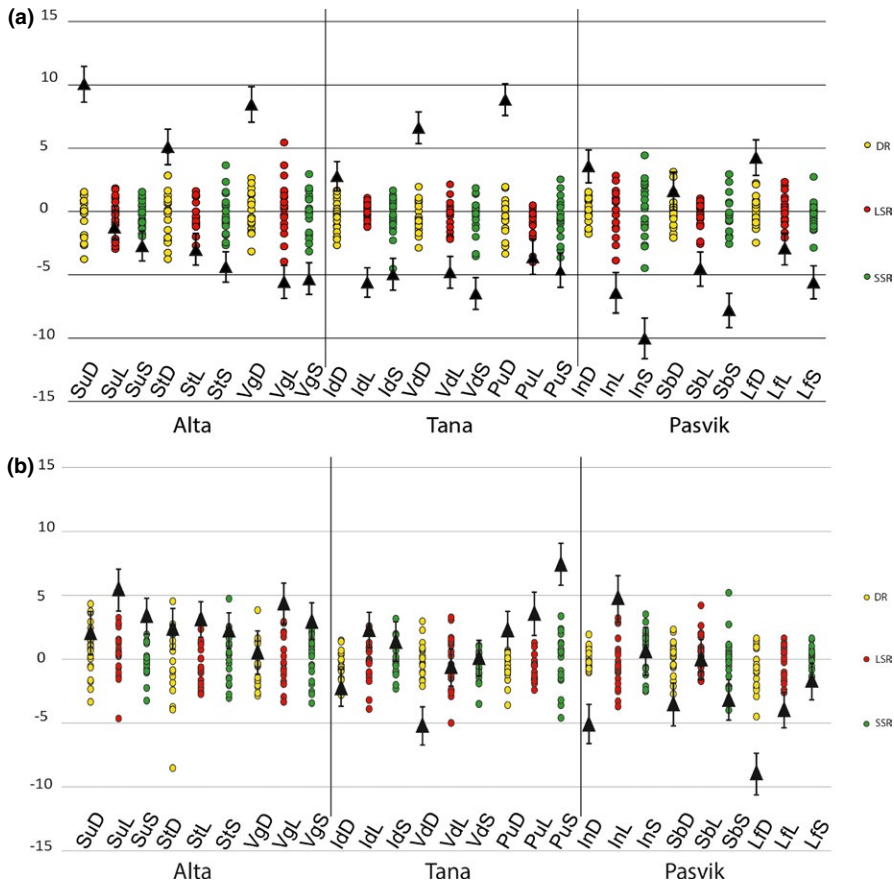


**FIGURE 3** Observed divergence of phenotypic traits in all study lakes. Each ellipse represents the drift distance for the population of same color. Observed divergence in study lakes and populations (see abbreviations from Table 2). The position of the population codes represents population-specific level of additive genetic effects, population means. The mean phenotype of each population is plotted together with estimated ancestral mean (A) and expected divergence under random genetic drift (ellipses). Each ellipse represents the drift distance for the population of the same color. The ellipses have different sizes, because the local populations experience different amounts of random genetic drift. Populations with mean value outside of their ellipses indicate divergent selection whereas populations with mean value inside the ellipse are expected to differentiate from ancestral population as a consequence of random drift

Fennoscandian whitefish morphs has been hypothesized to be the outcome of incipient ecological speciation toward three main lake habitats (e.g., Harrod et al., 2010; Østbye, Næsje, et al., 2005; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2010), where the numbers of gill rakers and body size of the fish have been proposed as potential key adaptive traits (Kahilainen & Østbye, 2006; Præbel, Knudsen, et al., 2013). The repeated occurrence of similar morph types throughout the northern Fennoscandia has also been suggested to be the product of parallel evolution (Østbye et al., 2006). This implies that these key traits repeatedly have been subjected to diversifying selection, as it is not likely that the traits would have repeatedly evolved by random drift alone. However, no other study has addressed the effect of random drift versus selection at a landscape level (but for lake level, see Præbel, Knudsen, et al., 2013).

Morphological adaptation to a specific niche has been investigated in a range of species, for example, head morphology in ecomorphs of Arctic charr (Adams et al., 1998; Recknagel et al., 2017). In European whitefish, the divergence in number and the length of gill rakers, head morphology, and body shape have been extensively studied (e.g., Amundsen et al., 2004; Hudson, Lundsgaard-Hansen, Lucek, Vonlanthen, & Seehausen, 2017; Kahilainen & Østbye, 2006; Siwertsson, Knudsen, Adams, et al., 2013; Siwertsson, Knudsen, Præbel, et al., 2013), where number and the length of gill rakers have

been identified as one of the most important niche-related morphological adaptations (Roesch, Lundsgaard-Hansen, Vonlanthen, Taverna, & Seehausen, 2013). Morphological differences in gill rakers affect the foraging efficiency (Roesch et al., 2013; Sanderson, Cheer, Goodrich, Graziano, & Callan, 2001) and have shown to be correlated with habitat choice and/or prey selectivity in a range of fish species, such as cichlids (Muschick et al., 2014), alewives (*Alosa pseudoharengus*; Post, Palkovacs, Schielke, & Dodson, 2008), and sticklebacks (Schluter & McPhail, 1992). However, the mechanism behind this phenotype-environment association is still not completely understood. In European whitefish from subarctic lakes, the number of gill rakers is positively correlated with the use of the pelagic habitat and the proportion of zooplankton in the diet (Kahilainen et al., 2011). As resource availability and prey size vary between the three principal habitats (Hayden, Harrod, & Kahilainen, 2014), similar habitats appear to produce phenotypes that are adapted to utilize habitat specific resources. In the pelagic habitat, the higher number of gill rakers facilitates consumption of small zooplankton (Roesch et al., 2013), whereas such gill rakers are not efficient for foraging larger benthic prey buried in sediment or sand (Lundsgaard-Hansen, Matthews, Vonlanthen, Taverna, & Seehausen, 2013). In order to enable indigestible particles to exit, the feeding of large benthic prey is correlated with a smaller number of short and robust gill rakers (Kahilainen et al., 2011). Accordingly,



**FIGURE 4** Population means in gill raker (a) and total length (b) traits. Triangular dots with error bars represent population means from the real data with 95% credibility interval. Small dots represent the amount of variation expected under drift-based divergence. Zero level is the ancestral mean. In this picture, there are 20 simulated replicates representing different scenarios (see morph and lake abbreviations from Table 2).

we observed statistically significant differences in the number of gill rakers between whitefish morphs increasing from profundal to littoral to pelagic morphs in all watercourses, where the observed mean number of gill rakers correlated with the niche use observed in other studies of whitefish (e.g., Amundsen et al., 2004; Harrod et al., 2010). Similar pattern of diversifying selection between niche uses is also found among other fish species, for example, Arctic charr and three-spined sticklebacks, in postglacial lakes (Skúlason, Snorrason, Ota, & Noakes, 1993; Taylor, 1999). Previous studies of lake whitefish and European whitefish species have revealed deviation from neutral expectation, suggesting that the number of gill rakers in whitefish has evolved as a product of natural selection (Østbye, Næsje, et al., 2005; Præbel, Knudsen, et al., 2013; Rogers et al., 2002). Our study confirms and expands these findings showing that natural selection induces distinct number of gill rakers in littoral, pelagic, and profundal morphs of European whitefish regardless of the repeated founder events. This was evident, as the observed divergence in this trait among habitats consistently was larger than the expected divergence under random genetic drift. There was furthermore consistent sign of a colonization history ranging from east to west in the ecological, phenotypic, and genetic divergence, especially toward the profundal habitat. The profundal SSR morph showed the most pronounced differences in body shape, number of gill rakers, habitat, and diet from the other benthic morph, the littoral LSR whitefish, in the oldest Pasvik watercourse populations compared to younger Alta populations (Harrod et al., 2010; Kahilainen et al., 2003; Siwertsson, Knudsen, Præbel, et al.,

2013). This was also in line with the observed genetic differentiation, where the level of reproductive isolation between the littoral and profundal whitefish morphs closely followed the ecological and phenotypic divergence (this study, Præbel, Knudsen, et al., 2013; Siwertsson, Knudsen, Præbel, et al., 2013) and suggests still ongoing ecological driven divergence in the westernmost Alta watercourse. In our study, the SSR whitefish in Alta and Tana watercourses showed significant signs of selection, although the population mean in number of gill rakers was not very far from the expected drift-based divergence. This may be due to shorter evolutionary time for divergence, differences in selection pressure between watercourses (e.g., lake depth, prey resources, and predation pressure), and/or the populations' potential to respond to selection (Nosil, Harmon, & Seehausen, 2009).

We observed a small antagonistic relationship between gill raker number and total length of fish. Feeding on zooplankton is usually associated with larger number of gill rakers, small body size, and slower growth as an effect of lower energy content and high population density, compared to benthic feeders (Kahilainen et al., 2003, 2005; Link & Hoff, 1998). Small body size and early sexual maturation of DR whitefish are also likely life-history adaptations to high predation-induced mortality, as this morph is the main prey for piscivorous fish such as pelagic brown trout (Jensen et al., 2008; Kahilainen & Lehtonen, 2003). For the other morphs, predation mortality is much lower: LSR whitefish is able to reach a size refuge from the gape size of piscivorous fish (Bøhn, Amundsen, Popova, Reshetnikov, & Staldvik, 2002), whereas SSR whitefish utilizes the dark profundal habitat with very

low amount of predators (Kahilainen & Lehtonen, 2003). However, predation-induced mortality is likely dependent on the abundance of piscivorous fish. In the Pasvik and Tana lakes with abundant and diverse predator populations, the DR whitefish were indeed smaller in size than the other two morphs. On the contrary, there were no significant differences in fish length between morphs in Alta. However, the mean body size of populations is dependent not just on the genes each individual possesses but also on environmental aspects such as prey availability and density-dependent resource competition (Kahilainen et al., 2003, 2005; Muir et al., 2010). As the heritability estimates for length are smaller than for the number of gill rakers, we have to take into account that phenotypic plasticity may have a larger effect on the length than on the number of gill rakers.

The buildup of reproductive isolation plays an important role in the speciation process. Populations that experience ecologically based diversifying selection are also subjected to diminishing amount of gene flow between them, which leads to development of reproductive isolation. One approach to assess how far the speciation process has proceeded is to measure the reproductive isolation between populations, as multiple studies have detected association between reproductive isolation and ecological divergence (e.g., Funk, Nosil, & Etges, 2006; Hendry, 2004; Lu & Bernatchez, 1999). An examination of the coancestry matrix obtained herein revealed different scenarios of relatedness and population structure between the three watercourses. In the Tana watercourse, all the study lakes appeared to form their own cluster, and there was little relatedness between the three study lakes that were located far apart. In the Pasvik watercourse, relatedness between whitefish populations was in general smaller than in the Alta watercourse, where especially the DR whitefish clustered together and had high interpopulation coancestry among them. The whitefish populations shared some level of genetic similarity, not just among morphs (across lakes), but also within different lakes. This may refer to parallel divergence in the same direction across lakes, even when the reproductive isolation between morphs is weak (see also Johannesson, 2001).

The observed phenotypic diversification in gill raker number has been proposed to have a recent origin (Østbye, Bernatchez, et al., 2005). Rapid response to selection is possible when the trait has a strong (additive) genetic basis and enough genetic variation at the initial stages of divergence (Hirsch, Eckmann, Oppelt, & Behrmann-Godel, 2013; Kopp & Matuszewski, 2014). Selection pressure and gene flow between subpopulations have effect on how fast the divergence builds up (Hendry, Wenburg, Bentzen, Volk, & Quinn, 2000). If there is substantial amount of gene flow between populations, the divergence may not arise as gene flow works against segregation, especially in a case of multifarious selection, where selection is operating on multiple genetically independent traits (Nosil, Harmon, et al., 2009). Gene flow may therefore slow down or prevent local adaptation and the formation of discrete populations (Blanquart, Gandon, & Nuismer, 2012; Nagylaki & Lou, 2008; Slatkin, 1985). Drift may contribute to the divergence even under conditions of strong natural selection. When the level of gene flow between populations is high, drift may overrun the joint effect of selection and gene flow, as selection and migration may reverse

each other's effect (Savolainen, Lascoux, & Merilä, 2013). In our study, the phenotypically and genetically most divergent whitefish populations were found in the oldest Pasvik watercourse, whereas the whitefish populations in the Alta watercourse were more influenced by drift due to founder effects. Notwithstanding, the three morphs have still evolved in all three watercourses, but the radiations appear to be at different stages of divergence. As the divergence likely have happened repeatedly in each lake (Østbye et al., 2006), "the starting gene pool" for the divergence process has most likely not been the same among watercourses. Nevertheless, we detected signals of selection also in the younger whitefish populations in the Alta watercourse. Labonne et al. (2016) demonstrated how selection may actively work to increase genetic variation, even in a case of strong founder effect and minimal genetic variation. Thus, reduced genetic variation does not necessarily prevent adaptation, as small populations may still retain their adaptive potential (Wood, Tezel, Joyal, & Fraser, 2015). However, when selection is operating with smaller degree of genetic variation, it may require a longer evolutionary time for niche differentiation, adaptation, and ecological speciation (Gavrilets, 2004; Hendry, 2009). Although the pelagic DR whitefish is found across all three watercourses, the substantially diverged SSR whitefish is currently present only in the older watercourses. Previous studies have proposed the existence of distinct European whitefish morphs as a result of parallel evolution (Østbye et al., 2006; Præbel, Knudsen, et al., 2013; Siwertsson, Knudsen, Adams, et al., 2013). The existence of a single mtDNA lineage and genetic clustering of morphs in their respective lakes or watercourses gives support to this hypothesis (Østbye, Bernatchez, et al., 2005). The possibility of neutral divergence due to random genetic drift is considered as an unlikely explanation for the repeated occurrence of phenotypically differentiated morphs in the three main habitats in many lakes across Fennoscandia. However, no previous studies have tested this hypothesis using a large landscape level dataset as in the current study. We observed parallel pattern of divergence across lakes, while the degree of divergence varied between watercourses. When assessing lakes separately, with different amount of standing genetic variation, it is likely that selection has been working with a different set of genetic variation in each lake.

To conclude, we have presented new results on the interplay between diversifying selection and random genetic drift in the evolution of local adaptation. Our results show that natural selection has worked toward stronger phenotype-environment correlations for the size of the fish and the number of gill rakers, where especially the gill raker number of a whitefish morph is an adaptation toward a more efficient use of the specific lake habitat. Further studies are necessary to understand the genetic mechanisms behind the diversification, and to what extent and how traits evolve at different levels of standing genetic variation.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

KP conceived the study; KH, KØ, and KP designed the study; KH, KKK, KP, and P-AA collected the samples; KH and KP performed the analysis; KH drafted the article; and all authors contributed critically to the revisions and gave final approval for publication.

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#### SUPPORTING INFORMATION

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# Allochrony as a potential driver for reproductive isolation in adaptive radiations of European whitefish ecomorphs

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## Abstract

In northern Fennoscandian lakes, monophylogenetic lineages of postglacial fishes are radiating into several adaptive forms, but the speciation process is still at an incipient stage. The speciation process has received increased attention over the years, but the underlying mechanisms and drivers are still debated and poorly understood. European whitefish (*Coregonus lavaretus* [L.]) is the most abundant fish species in these lakes and has evolved into several ecomorphs adapted to different trophic niches and habitats. Genetic divergence has been observed among these ecomorphs, but the mechanism(s) responsible for the ongoing build-up of reproductive isolation has still to be revealed. As these systems are young in evolutionary time (<10 kyr), prezygotic and postzygotic extrinsic isolation mechanisms are thought to be more likely to contribute to the reproductive isolation than intrinsic isolation mechanisms. We determined the gonadosomatic index (GSI) of three ecomorphs in two replicated lake systems and used GSI as a proxy to investigate the prezygotic isolation mechanism, allochrony, as a driving factor of divergence in this adaptive radiation of whitefish. We found that the three ecomorphs differed in GSI values within and between lakes, suggesting different spawning times of the ecomorphs. We also show that males of one ecomorph had equal onset of maturity as another ecomorph, giving novel insights into the ongoing gene flow observed between ecomorphs. The result supports allochrony as a driver for the divergence process of whitefish ecomorphs, but more evidence is still needed to rule out that the three ecomorphs make use of different spawning grounds.

## KEYWORDS

allochrony, *Coregonus lavaretus*, ecological speciation, gonadosomatic index, habitat preference, spawning time, whitefish

## 1 | INTRODUCTION

Adaptive radiation is the rapid evolutionary divergence of individuals from a common ancestor into a variety of adaptive forms (Futuyma, 1998) that exploit different ecological niches (Grant & Grant, 2008). Understanding what processes drive and maintain an adaptive radiation is a central question in evolutionary ecology. The availability of various ecological opportunities in an ecosystem

allows for different niches that may favour different behavioural and morphological adaptations. The association between a particular morphology and a specific niche is recognised as an important factor in adaptive radiation (Schluter, 2000), and in fish, this association is often related to foraging traits, for example head shape and trophic niche (Schluter, 1996). These associations can lead to genetic divergence and reproductive isolation of different adaptive forms (Rundle & Nosil, 2005; Schluter, 2000). Prezygotic and postzygotic isolations



are different isolation mechanisms that can result in reproductive isolation. Prezygotic isolation involves spatial and temporal isolation (e.g. different spawning sites and time) and sexual selection (Ritchie, 2007; Taylor & Friesen, 2017), while postzygotic isolation includes ecological inviability (extrinsic), hybrid inviability and sterility (intrinsic) (Coyne & Orr, 2004). Despite extensive efforts in understanding why and how reproductive isolation accumulates in adaptive radiations, the exact drivers still remain to be identified for many species.

Polymorphic populations are commonly found in several freshwater fish species in postglacial lakes of the Northern Hemisphere. The fish species inhabiting these lakes have shown rapid (<10 kyr), convergent phenotypic divergence and adaptive radiation into multiple ecomorphs (Häkli, Østbye, Kahilainen, Amundsen, & Præbel, 2018; Østbye et al., 2006; Østbye, Bernatchez, Næsje, Himberg, & Hindar, 2005; Schluter, 2000; Taylor, 1999). European whitefish (*Coregonus lavaretus* [L.], referred to as whitefish further on) is a widely distributed fish species in Europe and is highly abundant in northern Fennoscandia. Here, it has diverged from a monophyletic lineage (Østbye, Bernatchez, et al., 2005) into distinct ecological morphs through adaptive radiation (Østbye et al., 2006; Svärdson, 1979). Three distinct ecomorphs of whitefish have been described (Kahilainen & Østbye, 2006; Siwertsson et al., 2010), which show clear differences in niche utilisation and trophic morphology, for example head shape and gill rakers (Amundsen, Bøhn, & Vaga, 2004; Harrod, Mallela, & Kahilainen, 2010; Kahilainen & Østbye, 2006), and have also been found to differentiate genetically (Præbel, Knudsen, et al., 2013; Siwertsson et al., 2013). However, the reproductive isolation among the ecomorphs is not complete, evidenced by the frequent occurrence of hybrids between the ecomorphs (Bhat et al., 2014).

The three whitefish ecomorphs are associated with the feeding niches found in three main habitats of the lakes; the littoral, pelagic and profundal zones (Kahilainen, Lehtonen, & Könönen, 2003; Østbye et al., 2006). The ecomorphs are named according to morphology of gill rakers (Kahilainen & Østbye, 2006). The densely rakered whitefish ecomorph (hereafter DR whitefish) resides mainly in the pelagic habitat and is a zooplanktivorous specialist, the large sparsely rakered whitefish (LSR whitefish) mainly feeds on benthic macroinvertebrates in the littoral habitat, and the small sparsely rakered whitefish (SSR whitefish) is mainly found in the profundal habitat feeding on benthic invertebrates (Harrod et al., 2010). In a recent study, it was found that initial divergence of the ecomorphs into different habitats was a result of the presence of pike (*Esox lucius*) in the lakes (Öhlund, G., Bodin, M., Nilsson, K.A., Öhlund, S.-O., Mobley, K.B., Hudson, A.G., Peedu, M., Brännström, Å., Bartels, P. Præbel, K., Hein, C.L., Johansson, P., & Englund, G. unpublished). The presence of pike very likely has caused the whitefish to either refuge into new, but less suitable habitats, or maximise growth to reach a safe size. Apart from variation in food resources and basal sources of energy in the three principal habitats (Harrod et al., 2010), they also differ with regard to their thermal conditions (Evans, Præbel, Peruzzi, Amundsen, & Bernatchez, 2014; Hayden, Harrod, & Kahilainen, 2013). Thermal stratification of the water column in postglacial lakes means that littoral and pelagic habitats undergo large seasonal

changes in water temperature from 10–20°C in the warm summer months and to 0–2°C in the cold winter months when covered with ice (Hayden et al., 2013), whereas the profundal habitat display a cold, but stable, temperature of 2–8°C, throughout the year.

The isolation mechanism driving the ongoing divergence in adaptive radiation of whitefish still has to be identified in these northern systems. This is important for understanding the biological, ecological and genetic mechanisms involved in their adaptive radiation. Studies on whitefish in more southern Swedish lakes (Öhlund G., Bodin, M., Nilsson, K.A., Öhlund, S.-O., Mobley, K.B., Hudson, A.G., Peedu, M., Brännström, Å., Bartels, P. Præbel, K., Hein, C.L., Johansson, P., & Englund, G. unpublished, Svärdson, 1979) suggested spatial and temporal differences in spawning time, but little is known about the exact spawning times and places for the different ecomorphs in northern Fennoscandian lakes. Based on field observations in Lake Paadar of spawning shoals in shallow water, there were indications of the DR whitefish being the first ecomorph to spawn, followed by the LSR whitefish and finally SSR whitefish (K. Kahilainen pers. obs. in Kahilainen, Patterson, Sonninen, Harrod, & Kiljunen, 2014). A fourth ecomorph, LDR, is also present in Lake Padaar but it occurs in low abundance and is only present in the Pasvik watercourse, not the Alta watercourse where this study was performed. In coregonids, interspecific variation in spawning time can be an important factor driving reproductive isolation (Bernatchez et al., 2010; Hudson, Vonlanthen, Müller, & Seehausen, 2007; Svärdson, 1979). The late spawning time of SSR whitefish may reflect the dark and stable cold temperatures in profundal habitats which slow down metabolic processes (Ohlberger, Mehner, Staaks, & Hölker, 2008). Physiological adaptation, such as metabolic and maturation processes, to a particular light and thermal regime should differ substantially among the ecomorphs, but so far only niche-driven and respiration adaptations have been suggested as drivers of phenotypic divergence and reproductive isolation in northern postglacial lakes (Evans et al., 2014; Harrod et al., 2010; Keller & Seehausen, 2012; Østbye et al., 2006).

In this study, we investigated the prezygotic isolation mechanism(s) among the three whitefish ecomorphs. The challenge of revealing reproduction in these northern systems, compared with more southern lakes for example in Sweden, Denmark and the European Alps, is that the spawning takes place in late October to December, when the lake ice is too thin to work on, but too thick for boating. Based on our own field observations, information from locals and the presence of hybrids between the ecomorphs (Bhat et al., 2014), it seems that all three ecomorphs mainly share the same spawning ground(s) in the littoral zone, and with the knowledge of whitefish ecomorphs' thermal niches, we hypothesised that differences in spawning time (allochryony) acts as the main driver of reproductive isolation. This differs from the spatial divergence in spawning grounds observed in other more southern whitefish systems (Østbye, Næsje, Bernatchez, Sandlund, & Hindar, 2005; Vonlanthen et al., 2009). We sampled European whitefish ecomorphs in two replicated northern lakes, as close to their natural spawning time as possible, and calculated a gonadosomatic index (GSI) as a measurement of sexual maturity. GSI has previously been used to determine sexual maturity, seasonal changes and reproduction timing in

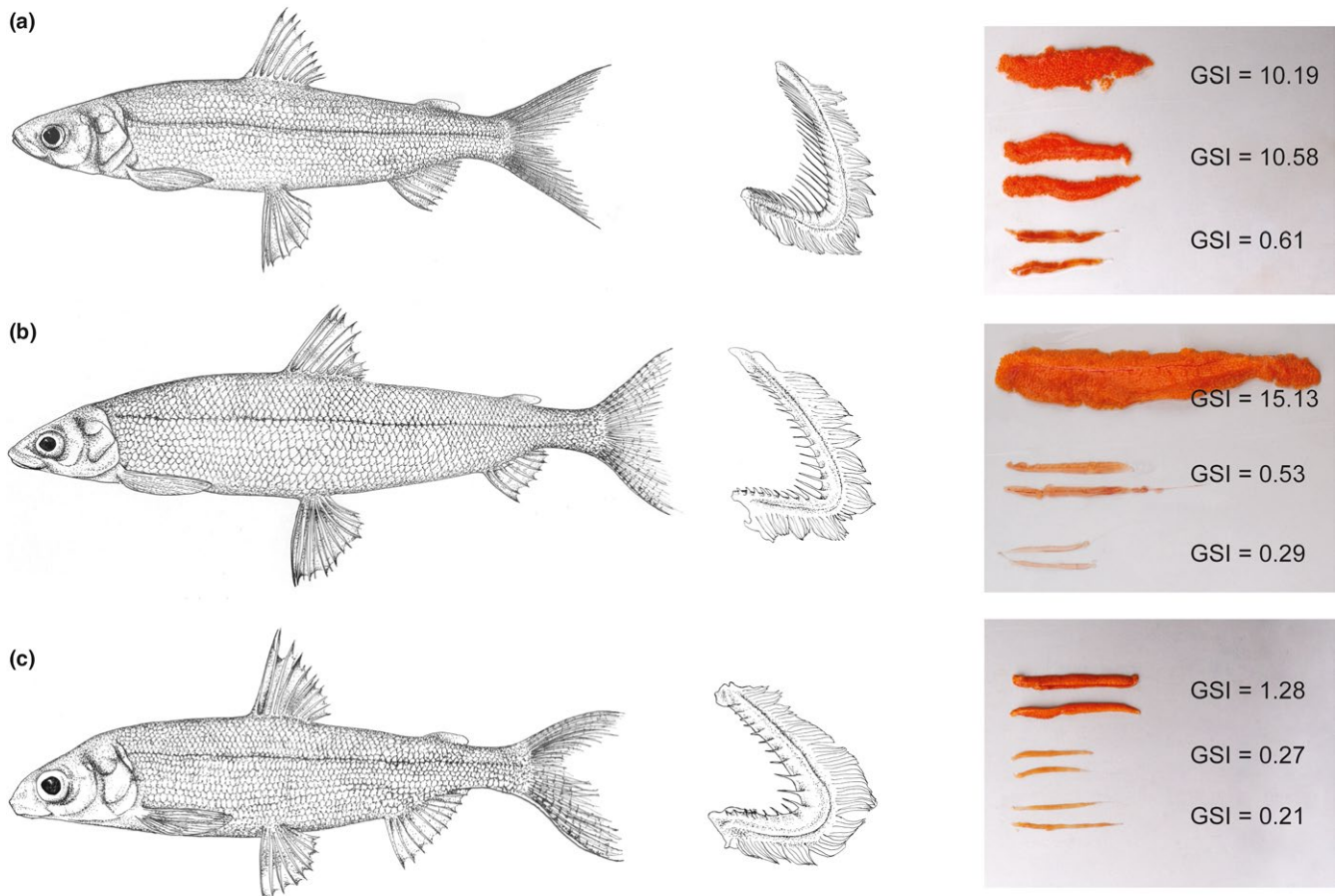
fish (Flores, Wiff, & Díaz, 2015; McQuinn, 1989; Valdés et al., 2004). Seasonal changes in GSI of female Blaufelchen whitefish (of a morph equivalent to the DR morph in this study) in Lake Constance-Obersee, Germany, showed that the GSI increased steadily over the summer and autumn until it reached a maximum, just before spawning in the winter (Rösch, 2000). To our knowledge, no previous study has used GSI to investigate allochry in a polymorphic species, and by comparing sexual maturation of the three whitefish ecomorphs, our goal was to infer whether the whitefish ecomorphs display differences in spawning time.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

In October 2016, over a period of seven days, two lakes located in the northern Fennoscandia were sampled for European whitefish ecomorphs, Lake Stuorajávri (69°06'N, 22°49'E) and Lake Suohpatjávri (68°56'N, 23°05'E). Stuorajávri covers an area of 24 km<sup>2</sup> with a maximum depth of 30 m, and Suohpatjávri covers 2 km<sup>2</sup> with a maximum depth of 25 m. The two lakes are oligotrophic,

harbour six fish species in addition to the whitefish and have relatively equal distribution of shallow and deep areas. Both lakes have three principal habitats: the littoral habitat (shore water, close proximity to the bottom, <10 m depth; >1% of light at surface), the profundal habitat (close proximity to the bottom, >10 m depth; <1% of light at surface) and the pelagic habitat (open water, 0–6 m depths). Three different ecomorphs of whitefish are found in both of these lakes: DR, LSR and SSR whitefish (Siwertsson et al., 2013) (Figure 1). Fish sampling was performed with standardised gillnets of different mesh sizes, 10, 12.5, 15, 18.5, 22, 26, 35 and 45 mm. Benthic gillnets (1.5 m high) were used in the littoral and profundal zones, whereas the pelagic habitat was sampled using 6-m-high floating nets. A total of 364 whitefish were collected. For each individual fish, total body weight and gonad weight were determined in the field laboratory using a Sartorius BP 310s scale and subsequently used to calculate the gonadosomatic index (GSI = [testis or ovary weight/ body weight]\*100) per individual (Table 1). We further recorded the sex (female/male), sexual maturity (immature/mature) and total length for all individuals. Figure 1 shows a subsample of gonads for each whitefish ecomorph documented using a Nikon D610 camera body



**FIGURE 1** Drawings of the three ecomorphs of European whitefish (*Coregonus lavaretus* L.) from northern Norway and their respective gill rakers: (a) DR, densely rakered, (b) LSR, large sparsely rakered, and (c) SSR, small sparsely rakered ecomorph (line drawings modified from Harrod et al., 2010). The photographs show three examples of female gonads for each ecomorph at the exact same time of year in Suohpatjávri: DR (mature, mature, immature), LSR (mature, immature, immature) and SSR (mature, immature, immature). The GSI index values calculated as a measure of sexual maturity are provided next to the corresponding gonad

**TABLE 1** Overview of the whitefish dataset including average number of gill rakers  $\pm$  SD; number of individuals used in  $F_{ST}$ -calculations; number of mature individuals; average length, weight and gonad weight  $\pm$  SD; percentage of maturity; and average GSI for the three ecomorphs in Stuurajávri and Suohpatjávri, divided by sex

Ecomorph	Lake	Gill rakers (N)	$N(F_{ST})^a$	N (mature)	Length (mm)	Weight (g)	Gonad weight (g)	%Maturity	AverageGSI (g)
Females									
DR	Stuurajávri	34.8 $\pm$ 2.1	16	23	310 $\pm$ 22.75	236 $\pm$ 32.48	22.77 $\pm$ 5.37	85.2%	9.59 $\pm$ 1.34
	Suohpatjávri	39.0 $\pm$ 2.5	51	79	237 $\pm$ 23.52	89 $\pm$ 22.89	5.54 $\pm$ 2.53	97.6%	6.35 $\pm$ 3.05
LSR	Stuurajávri	24.4 $\pm$ 1.6	19	18	342 $\pm$ 48.67	317 $\pm$ 152.6	31.58 $\pm$ 34.51	94.7%	8.05 $\pm$ 8.09
	Suohpatjávri	26.5 $\pm$ 2.4	27	21	313 $\pm$ 40.49	245 $\pm$ 117.4	8.83 $\pm$ 14.04	63.6%	3.66 $\pm$ 4.85
SSR	Stuurajávri	22.0 $\pm$ 1.6	26	22	267 $\pm$ 42.37	154 $\pm$ 101.8	10.28 $\pm$ 17.31	75.9%	5.14 $\pm$ 4.38
	Suohpatjávri	23.6 $\pm$ 3.3	10	5	269 $\pm$ 7.92	134 $\pm$ 23.93	0.94 $\pm$ 0.52	50%	0.71 $\pm$ 0.34
Ecomorph	Lake	Gill rakers (N)	$N(F_{ST})^a$	N (mature) <sup>b</sup>	Length (mm)	Weight (g)	Gonad weight (g)	%Maturity	AverageGSI (g)
Males									
DR	Stuurajávri	34.8 $\pm$ 2.1	31	50	317 $\pm$ 13.26	241 $\pm$ 30.76	3.35 $\pm$ 0.90	100%	1.39 $\pm$ 0.33
	Suohpatjávri	39.0 $\pm$ 2.5	18	27	232 $\pm$ 24.65	85.5 $\pm$ 27.21	0.83 $\pm$ 0.34	100%	0.96 $\pm$ 0.15
LSR	Stuurajávri	24.4 $\pm$ 1.6	26	26	348 $\pm$ 28.46	331 $\pm$ 99.81	4.55 $\pm$ 2.40	93.1%	1.32 $\pm$ 0.29
	Suohpatjávri	26.5 $\pm$ 2.4	24	14	296 $\pm$ 42.35	207 $\pm$ 122	2.47 $\pm$ 2.14	67.9%	1.12 $\pm$ 0.31
SSR	Stuurajávri	22.0 $\pm$ 1.6	25	21	259 $\pm$ 32.79	131 $\pm$ 53.30	1.32 $\pm$ 0.71	85.2%	1.00 $\pm$ 0.22
	Suohpatjávri	23.6 $\pm$ 3.3	2	1 <sup>c</sup>	356 <sup>c</sup>	422 <sup>c</sup>	3.12 <sup>c</sup>	50% <sup>c</sup>	0.74 <sup>c</sup>

Note. Abbreviations: DR, densely rakered whitefish; LSR, large sparsely rakered whitefish; SSR, small sparsely rakered whitefish.

<sup>a</sup>Both mature and immature individuals were genotyped, but not all individuals. Therefore, the number of individuals can vary from the number of mature individuals used for the GSI calculations.

<sup>b</sup>Outlier males were removed from the data set and are therefore not included here. A total of 139 mature males. <sup>c</sup>Only one mature male SSR, therefore averages and percentages calculated for this ecomorph are not correct. We kept the values to display the one male we did include in the study.



mounted with a Nikon 16.0–35.0 mm f/4.0ED lens. A fin tissue sample was also collected and stored in 96% ethanol at  $-20^{\circ}\text{C}$  for later genetic analysis.

## 2.2 | Ecomorph assignment and discrimination

A phenotypic analysis of the fish was performed in the field, where each individual was classified as DR, LSR or SSR whitefish according to appearance, head and body shape, and a visual evaluation of the gill raker morphology (Amundsen et al., 2004; Kahilainen & Østbye, 2006). Ecomorph assignment was further verified in the laboratory by assessing the first left branchial arch under a microscope and counting the number of gill rakers (Table 1). We did not distinguish between male and female when counting of gill rakers.

To assess genetic divergence between the ecomorphs, we followed the protocol of Præbel, Westgaard, et al. (2013) using a panel of 22 microsatellite markers. The data were screened for scoring errors, allele dropouts and null alleles using Micro-Checker 2.2.3 (van Oosterhout, Hutchinson, Wills, & Shipley, 2004). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using exact tests (Guo & Thompson, 1992) as implemented in GenePop 4.0 (Rousset, 2007). Sequential Bonferroni corrections (BFC) were used to correct pairwise comparisons for multiple comparisons following Rice (1989). Genetic divergence among morphs was estimated using the pairwise.fst function in the Aegenet R-package (Jombart, 2008), which computes Nei's estimator of pairwise  $F_{ST}$ , and tested for significance using 1,000 permutations. For the genetic divergence analysis, we used both immature and mature individuals.

## 2.3 | Preliminary analyses and quality control of GSI maturity data

For each lake, fish were divided into groups based on ecomorph, sex and sexual maturity (immature and mature). Division of sex was necessary because the weight of the gonads differs greatly between males and females, and the assessment of sexual maturity was necessary because we only were interested in using mature individuals for this study. Individuals that did not fulfil these criteria were excluded from the analyses, leaving 168 females and 148 males for the statistical analyses. Gonadosomatic index was calculated and used as a measurement for sexual maturity, that is the advancement of sexual maturity towards spawning, because all individuals were mature. This GSI maturity functioned as our proxy for differences in spawning time between whitefish ecomorphs. This means that the GSI values are under the assumption that greater GSI values equals a more sexually mature fish, and consequently, has an earlier spawning time, than a fish with a lower GSI value. The GSI values were transformed using the logarithmic function to more closely meet the assumption of linear models and because our dependent variable is a proportion (Baum, 2008). An overview of the number of mature individuals, percentage of maturity and the average GSI for each ecomorph in the two lakes is found in Table 1.

## 2.4 | Determination of variation in GSI maturity among whitefish ecomorphs

For the statistical data analyses of GSI maturity among ecomorphs, we carried out a two-way analysis of variance (ANOVA) using R v.1.0.44 (R Core Team, 2016). The first step was model selection using the *MuMin* package in R (Bartoń, 2017) to find the linear model that best explained the differences in maturity of whitefish ( $\log_{10}(\text{GSI})$ ). Using a factorial design of relevant variables, we tested 16 different linear models. The additive model of two factors (or independent variables), population (ecomorphs) and lake, was the most parsimonious fit according to Akaike's information criteria (AIC) statistics for model selection. This model had, for both sexes, the lowest AIC value with the lowest degrees of freedom and most weight to it. Next step was a control of the model to check if the data fitted a normal distribution by plotting a Q–Q plot. To detect the presence of outliers in our dataset, we used the interquartile range rule (IQR). For the males only, the data contained nine outliers outside 1.5 x IQR that skewed the distribution and thus needed to be removed from the dataset. This left us with 139 males for further analysis. The nine outliers removed from the male dataset consisted of eight individuals with much lower GSI values, indicating they were wrongly assigned as sexually mature in the field when in fact they were immature. One individual had a much higher GSI value than average, which may be the result of a typo error during the field work. The gonad weight of this particular individual is >50% more than average. After the preliminary steps, the ANOVA statistics were performed and finally post hoc analyses using pairwise comparisons of least squares means for the specified factors in the linear model using the *estimability* and *lsmeans* packages in R (Lenth, 2016a, 2016b).

Additionally, we caught an unusually low number of SSR whitefish in Lake Suohpatjävri, only one mature male in total. This was clearly not enough to do proper statistical analysis, but we included the individual anyway because this individual was in fact not an outlier in the model control step allowing us to obtain some insight into the GSI values of male SSR whitefish.

## 3 | RESULTS

### 3.1 | Genetic differentiation among whitefish morphs

The number of alleles at each microsatellite locus ranged from 2 to 21 across populations (Table S1). Micro-Checker detected homozygote excess due to possible null alleles in four of 22 tested loci: *Coc1\_lav27* (Stuorajävri, DR whitefish), and *BWF1*, *Clatet06* and *Coc1\_lav10* (Stuorajävri, SSR whitefish) (Table S1). Although none of the possible null alleles were found in more than one population at a time, those four loci were excluded from further analysis. Deviations from HWE were indicated in one out of 108 tests (0.9%) after sequential Bonferroni corrections, which are less than expected by chance (5%). Out of 918 tests, significant linkage disequilibrium was found in three (0.3%) loci comparisons after Bonferroni correction. Pairwise  $F_{ST}$

values between populations were small yet statistically significant, ranging from 0.011 to 0.059, where the smallest genetic difference was found between LSR whitefish in different lakes (Table 2).

### 3.2 | Field-determined maturity of whitefish morphs

The abundance of mature individuals differed both between the ecomorphs and lakes (Table 1). For both sexes, we found a pattern that most often the DR whitefish had more mature individuals than the LSR whitefish, which again had more mature individuals than the SSR whitefish. The only exception being females in Stuurajávri, where 85.2% of DR whitefish were mature vs. the 94.7% of LSR whitefish (Table 1). Between lakes, there was a higher proportion of mature whitefish caught in Stuurajávri than in Suohpatjávri, especially for the LSR and SSR whitefish. The DR whitefish appeared to have an equal proportion of mature individuals between the lakes.

### 3.3 | Variation in GSI maturity of whitefish ecomorphs

The two-way ANOVA showed that there are significant differences in the advancement of sexual maturity ( $\log_{10}GSI$ ) between the three distinguished whitefish ecomorphs for both sexes (females:  $F_{2,165} = 17.359, p < 0.001$ ; males:  $F_{2,135} = 9.097, p < 0.001$ ). There were also significant differences in GSI maturity for both sexes between the two lakes (females:  $F_{1,165} = 15.293, p < 0.001$ ; males:  $F_{1,135} = 46.838, p < 0.001$ ). For the females, the DR whitefish were significantly more mature than the LSR whitefish ( $p < 0.001, R^2=0.2186$ ) and the SSR whitefish ( $p < 0.001, R^2=0.2186$ ), but the LSR whitefish were not significantly more mature than the SSR whitefish ( $p = 0.597, R^2=0.2485$ ). For the males, there was no difference in maturity between DR whitefish and LSR whitefish ( $p = 0.752, R^2=0.310$ ), but both the DR whitefish ( $p < 0.001, R^2=0.310$ ) and LSR whitefish ( $p < 0.001, R^2=0.2615$ ) were significantly more mature than the SSR whitefish.

For the post hoc analyses, we did pairwise comparisons using least square means of  $\log_{10}(GSI)$  on the specified factors in the linear model (Figure 2a,b). These analyses contrasted the advancement of sexual maturity of the whitefish ecomorphs individually from each

**TABLE 2**  $F_{ST}$  table of all genotyped individuals. Below diagonal are pairwise  $F_{ST}$  values, above diagonal are  $p$ -values

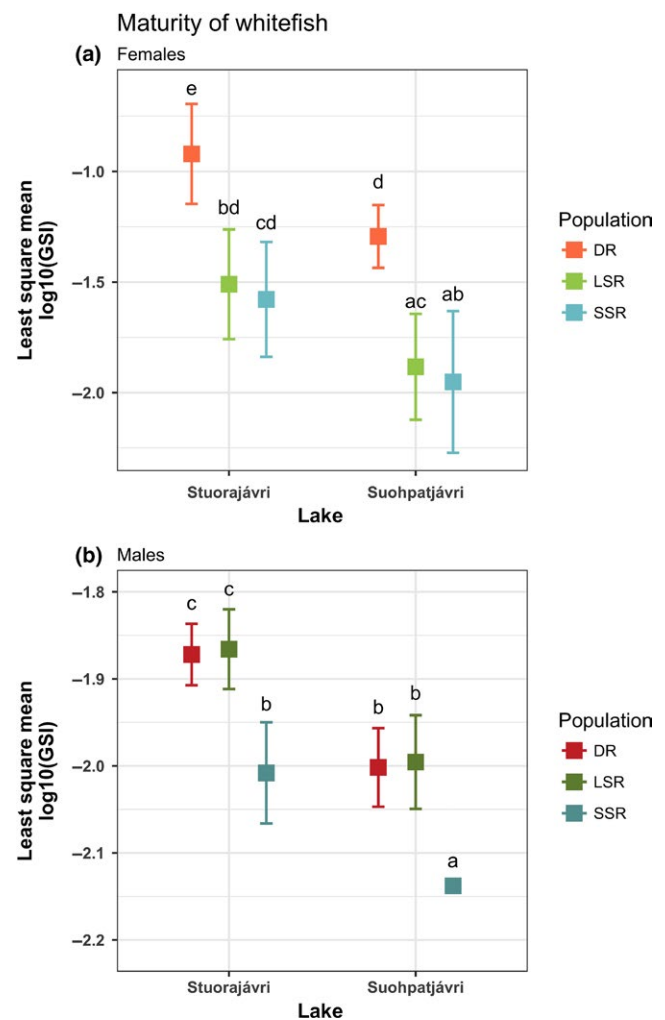
	StD	StL	StS	SuD	SuL	SuS
StD		0.001	0.001	0.014	0.001	0.001
StL	0.049		0.005	0.001	0.030	0.001
StS	0.035	0.016		0.001	0.005	0.008
SuD	0.012	0.059	0.042		0.001	0.001
SuL	0.037	0.011	0.017	0.047		0.008
SuS	0.038	0.020	0.016	0.032	0.014	

Note. Abbreviations of populations are the following: St (Stuurajávri), Su (Suohpatjávri), D (DR whitefish), L (LSR whitefish) and S (SSR whitefish). Code is a combination of lake and morph name.

lake. For the female whitefish, the whitefish from Stuurajávri were significantly more mature compared with whitefish in Suohpatjávri. Also, for females, the DR whitefish in both lakes were more mature compared with the LSR whitefish and SSR whitefish, which in turn were equally mature. For the male whitefish, as with the females, the whitefish in Stuurajávri were more mature compared with whitefish in Suohpatjávri. Furthermore, the male DR whitefish and LSR whitefish were equally mature, whereas they were significantly more mature than SSR whitefish in both lakes.

## 4 | DISCUSSION

We found statistically significant differences in the advancement of sexual maturity, based on GSI values, between the three genetically



**FIGURE 2** Plots showing the differences in maturity of the whitefish ecomorphs, for females (a) and males (b) and across two lakes. This was done using least square means for the specified factors in the linear model, and  $p$ -values were adjusted for multiple comparisons by Tukey's HSD. Boxes indicate the least square mean of  $\log_{10}(GSI)$ , and error bars indicate the 95% confidence interval of the least square mean. Means sharing a letter have no statistically significant difference at the  $\alpha$  level 0.05

and morphologically divergent ecomorphs of whitefish. We demonstrated that the pelagic DR whitefish were generally further advanced in sexual maturity than the littoral LSR whitefish, which in turn were more mature than the profundal SSR whitefish at the moment of capture. This pattern was prevalent in both of the sampled postglacial lakes and for both sexes. The pattern was also evident from the morphological appearance of the gonads of the ecomorphs, where DR whitefish had far more developed oocytes than the other ecomorphs at the moment of capture (Figure 1). However, our results do not permit inference about the temporal pattern of gonad development nor provide information about ecological factors that drive the maturation. Rösch (2000) investigated, over a five-year period, seasonal changes in GSI of the Blauefchen whitefish (morph equivalent to the DR morph in this study) in Lake Constance-Obersee, Germany, and found that GSI increased from low values in summer to about 24% of the body weight immediately before spawning in December. A similar result was obtained for Pollan whitefish in Lough Neagh, Ireland (Wilson & Pitcher, 1983), suggesting that GSI is a useful measure of sexual maturity in *Coregonus*. The whitefish radiations in northern Fennoscandia have been shown to be driven by ecological opportunity (Siwertsson et al., 2010), where each of the ecomorphs has adopted a life history and specialisation to each of the principal lake niches (littoral, pelagic, profundal). The DR whitefish feed on pelagic prey, which leads to the smallest body size and earliest sexual maturation, the LSR whitefish feed on littoral benthic macroinvertebrates, which leads to large size and late sexual maturation, and the SSR whitefish feed on profundal benthic prey and grow to intermediate body size and late sexual maturation (Harrod et al., 2010; Hayden et al., 2013; Kahilainen, Alajärvi, & Lehtonen, 2005; Kahilainen et al., 2003; Østbye et al., 2006). Our results of GSI-estimated maturity extend the understanding of the general life history characteristics of the ecomorphs by adding a likely spawning chronology, similar to what has been observed in the field on a single occasion (K. Kahilainen pers. obs. in Kahilainen et al., 2014).

Estimating the advancement of sexual maturity with GSI was based on the assumption that GSI values increase with increasing sexual maturity and earlier spawning time. According to Ohlberger's theory (2013), the temporal spawning segregation (allochry) develops because individuals living in different habitats experience different water temperatures and therefore mature at different rates. For whitefish, a discussed mechanism to drive differences in timing of spawning activities, and thus differences in sexual maturity between diverging ecomorphs, is the exposure to different environmental conditions, for example light and temperature regimes (Evans et al., 2014; Kahilainen et al., 2014). Adaptation to divergent thermal niches is also likely to be of crucial importance to the relative capacity to assimilate the energy required to reach sexual maturity and the development of gonads (Kahilainen et al., 2014). These habitat-specific differences reflect what the three whitefish ecomorphs in northern postglacial lakes experience, where the DR, LSR and SSR whitefish segregate in the pelagic, littoral and profundal habitats respectively (Østbye et al., 2006; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2013; Svärdson, 1979). The relative contribution of

ecological and physical factors on timing of sexual maturity for these whitefish ecomorphs remains to be investigated. But, all together, our hypothesis is supported in that the accumulation of genetic differences observed between the three whitefish ecomorphs (Østbye et al., 2006; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2013), is driven by a prezygotic extrinsic isolation mechanism, and is best explained by differences in spawning time, allochry.

The lakes, as local entries, also appear to have an influence on sexual maturity as we found statistically significant difference of the advancement of sexual maturity between the two lakes. The whitefish from Stuurajávri were significantly more advanced in sexual maturity than the whitefish from Suohpatjávri. Stuurajávri is larger (24 km<sup>2</sup>) and a little deeper (max depth of 30 m) compared with Suohpatjávri (2.0 km<sup>2</sup>, 25 m) and may therefore retain an overall warmer annual water temperature. Increased environmental temperatures are known to accelerate the physiological rate of animals and may induce earlier maturation of the Stuurajávri whitefish. Locals and scientists have also observed Stuurajávri whitefish to spawn in October–December (Pers. Com. Rune Knudsen) and in Suohpatjávri in November–January (Pers. Com. Eleonor Beck). However, further long-term studies are needed to elucidate the relative contribution of light and temperature to the extrinsic postzygotic isolation mechanisms in the northern Fennoscandian whitefish radiations to confirm these observations.

Where, when and how the whitefish spawn in nature is evidently important for understanding the evolution of these radiations. Earlier maturation of male whitefish and their presence on the spawning grounds before and after the arrival and spawning of females have been observed for Pollan whitefish in Lough Neagh, Ireland (Dabrowski, 1981). We also found, in both lakes, that male and female DR and male LSR whitefish displayed a similar state of maturity, while the female LSR and male and female SSR whitefish were less mature. This suggests that in these lakes, the temporal chronology of reproduction is as follows: DR whitefish, LSR whitefish and SSR whitefish. Notably, we show that the male LSR whitefish is mature at the same time as DR whitefish, suggesting a sex-specific route for the ongoing gene flow observed between the whitefish ecomorphs in their adaptive radiation (Bhat et al., 2014; Häkli et al., 2018; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2013). Hybrids between whitefish ecomorphs frequently occur in all northern Fennoscandian lakes, but genetic studies have only observed hybridisation between DR-LSR whitefish (Bhat et al., 2014) and between LSR-SSR whitefish (Præbel et al. unpublished). A study, using genome-wide coverage of SNPs to infer genomic consequences of speciation reversal in whitefish ecomorphs, showed that hybridisation between female DR and male LSR is the most likely route of gene flow between the whitefish ecomorphs (Bhat, 2016). The exact mechanism of this hybridisation is not known, and a potential explanation could be sneaking behaviour of LSR males, which has been shown in other salmonids (e.g. Baxter, Taylor, Devlin, Hagen, & McPhail, 1997; Garcia-Vazquez et al., 2001; Redenbach & Taylor, 2003). However, this behaviour has thus far only been described for small males and we therefore find it most likely to be caused by an overlap in spawning. Collectively,

the ongoing gene flow between whitefish ecomorphs may be explained by sexually mature male LSR whitefish that spawn with mature DR and we propose that also male SSR x Female LSR is a possible sex-specific route for gene flow between the SSR and LSR whitefish ecomorphs (Figure 2a,b). These results also support our hypothesis that the ecomorphs share spawning ground(s), but are reproductively isolated in time.

The most pronounced difference in terms of trophic ecology, morphology and reproductive isolation has been found between the SSR and DR whitefish (Harrod et al., 2010; Kahilainen & Østbye, 2006; Præbel, Knudsen, et al., 2013; Siwertsson et al., 2013). Our results also support these observations in both lakes and for both sexes. There was no overlap of GSI values between the DR and SSR ecomorphs indicating that extrinsic prezygotic isolation mechanisms are in play, quite possibly allochrony, and thus drive the diverging populations. Temporal separation can be important in achieving reproductive isolation in incipient ecological speciation (Schluter, 2001), where intrinsic postzygotic isolation mechanisms, such as genomic incompatibility, has yet to be accumulated between the diverging ecomorphs. A recent study by Johannsson, (2017) established that spawning window and population age were positively correlated in all ecomorphs of a lake. Hence, the older the lake is, the more prevalent divergence is for the whitefish ecomorphs. Overall spawning time is therefore increased as an intrinsic isolation mechanism to reduce contact and avoid interbreeding. This supports allochrony as a potential main driver for reproductive isolation between whitefish ecomorphs.

In conclusion, we found statistically significant differences in maturity between whitefish ecomorphs. Overall, at the moment of capture, the pelagic, zooplanktivorous DR whitefish displayed greater sexual advancement than the littoral, benthic feeding LSR whitefish, which again showed greater maturity than the profundal, benthic feeding SSR whitefish. This was the general pattern for both lakes, despite Stuurajávri showing greater proportion of mature individuals than Suohpatjávri. The morphological divergence between the ecomorphs, their adaptation to different thermal niches and sex-specific routes for gene flow between the ecomorphs, all suggest allochrony as a prezygotic isolation mechanism that contributes to the adaptive radiation in European whitefish.

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## AUTHOR CONTRIBUTIONS

KP and JBT conceived and designed the investigation. JBT, KH, SB and KP performed field and/or laboratory work, analysed the data

and wrote paper. JBT, KH and KP contributed materials, reagents and/or analysis tools.

## DATA AVAILABILITY STATEMENT

The data is available from the UiT Open Research Data, DOI: <https://doi.org/10.18710/CGZDTP>.

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