



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)

Name of candidate: **Katja Häkli**

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)

	Paper I	Paper II	Paper III
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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Paper I

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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

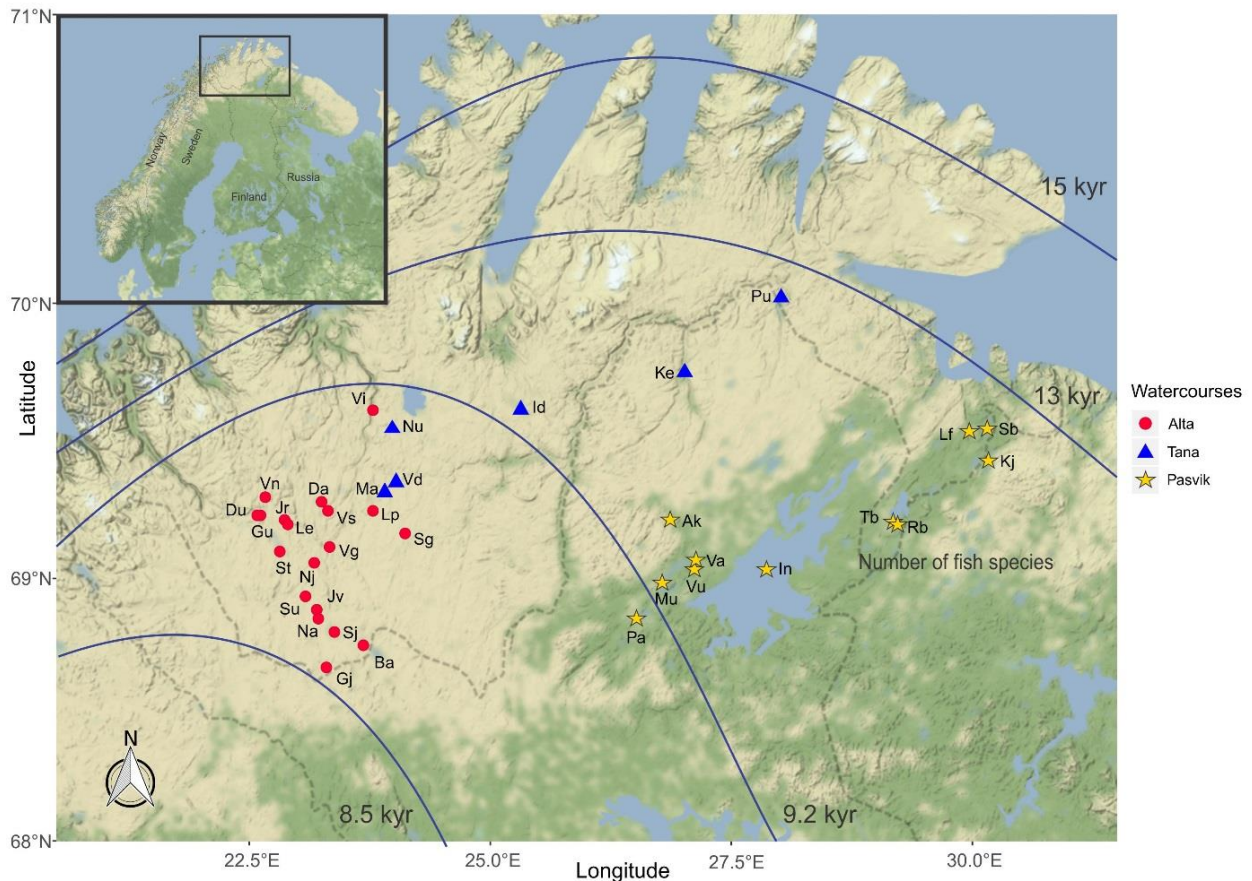


Figure 1. Map of the study area. Lakes in Alta, Tana and Pasvik watercourses are coded with red dots, blue triangles and yellow stars. Contour lines represent the retreating ice. Sampling sites are coded with abbreviations of lake names (see **Paper II**).

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.

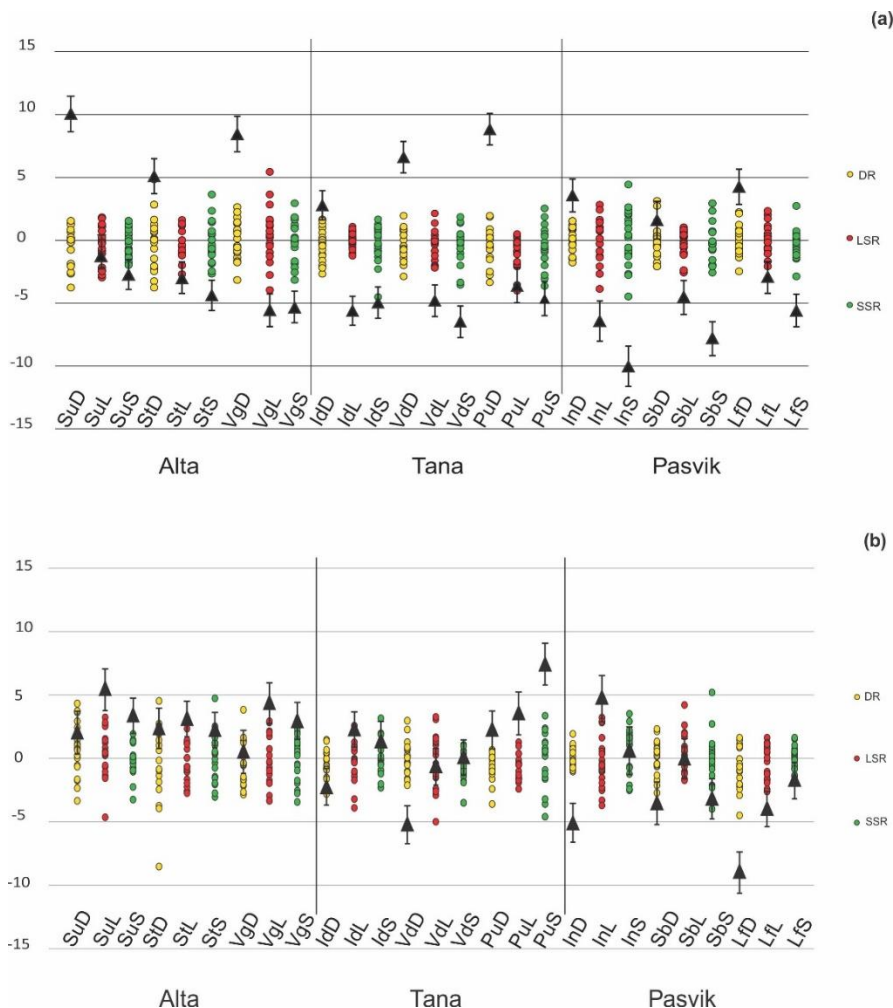


Figure 2. Population means in gill raker (a) and total length (b) traits. Triangular dots with error bars represent population means from the real data with 95% credibility interval. Small dots represent the amount of variation expected under drift-based divergence. Zero level is the ancestral mean. 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.

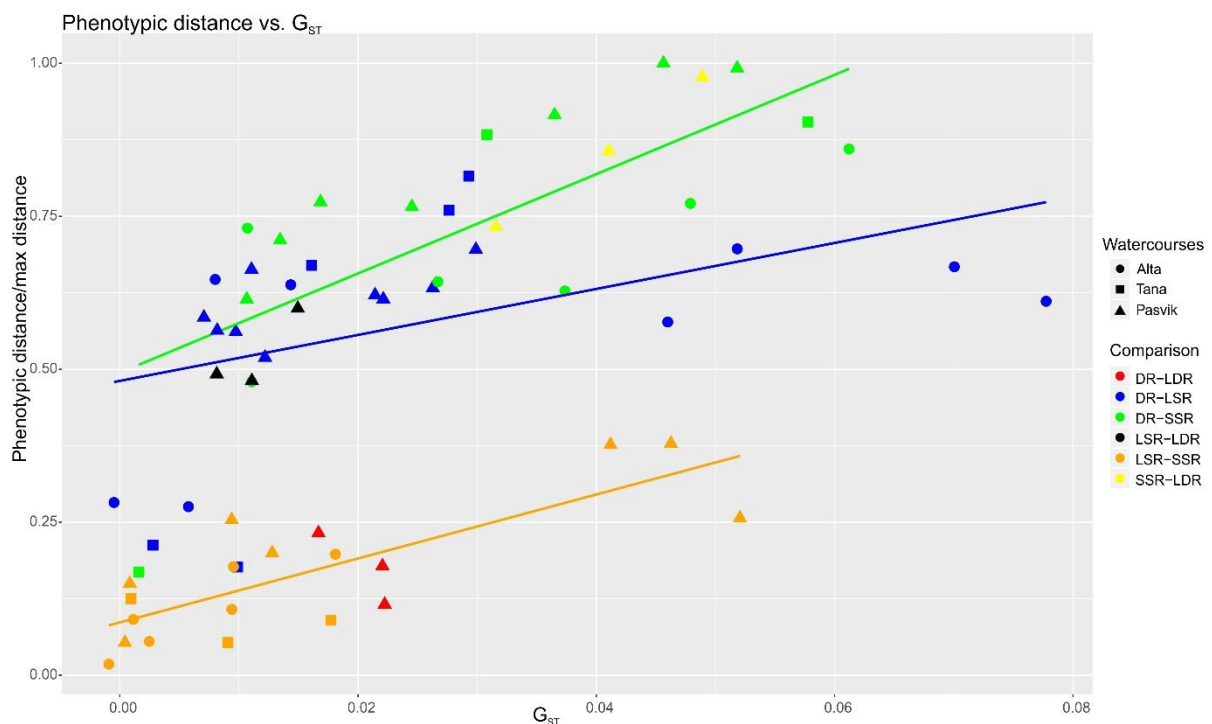


Figure 3. Pairwise comparison of G_{ST} and phenotypic distance between eco-morphs in each lake among watercourses. Different shapes represent the three watercourses, and morph wise comparisons in each lake are color-coded accordingly. Straight lines represents the regression of each eco-morph pair

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.

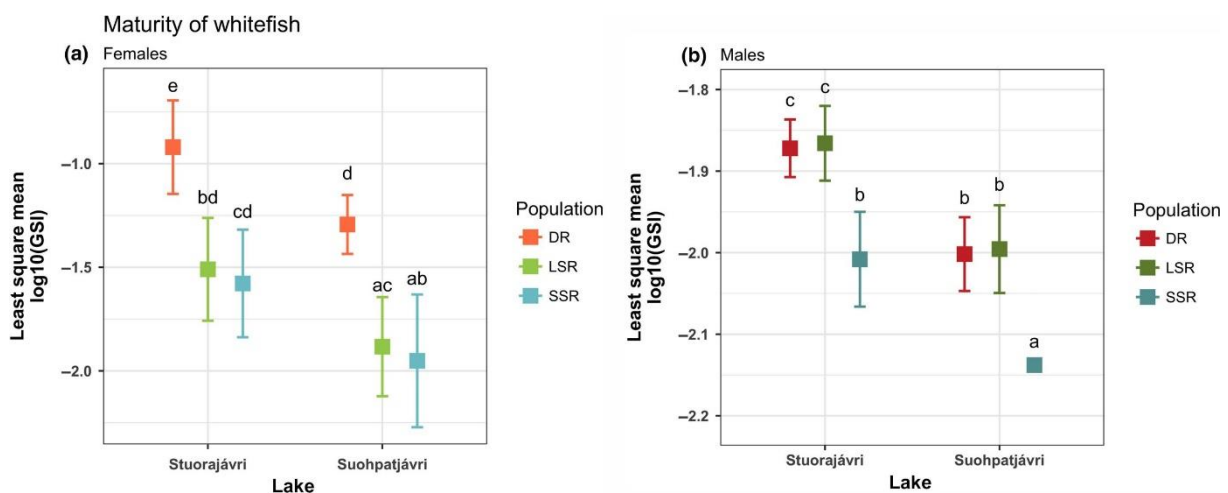


Figure 4. Plots showing the differences in maturity of the whitefish ecomorphs, for females (a) and males (b) and across two lakes. This was done using least square means for the specified factors in the linear model, and p-values were adjusted for multiple comparisons by Tukey's HSD. Boxes indicate 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, Stroeven 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.

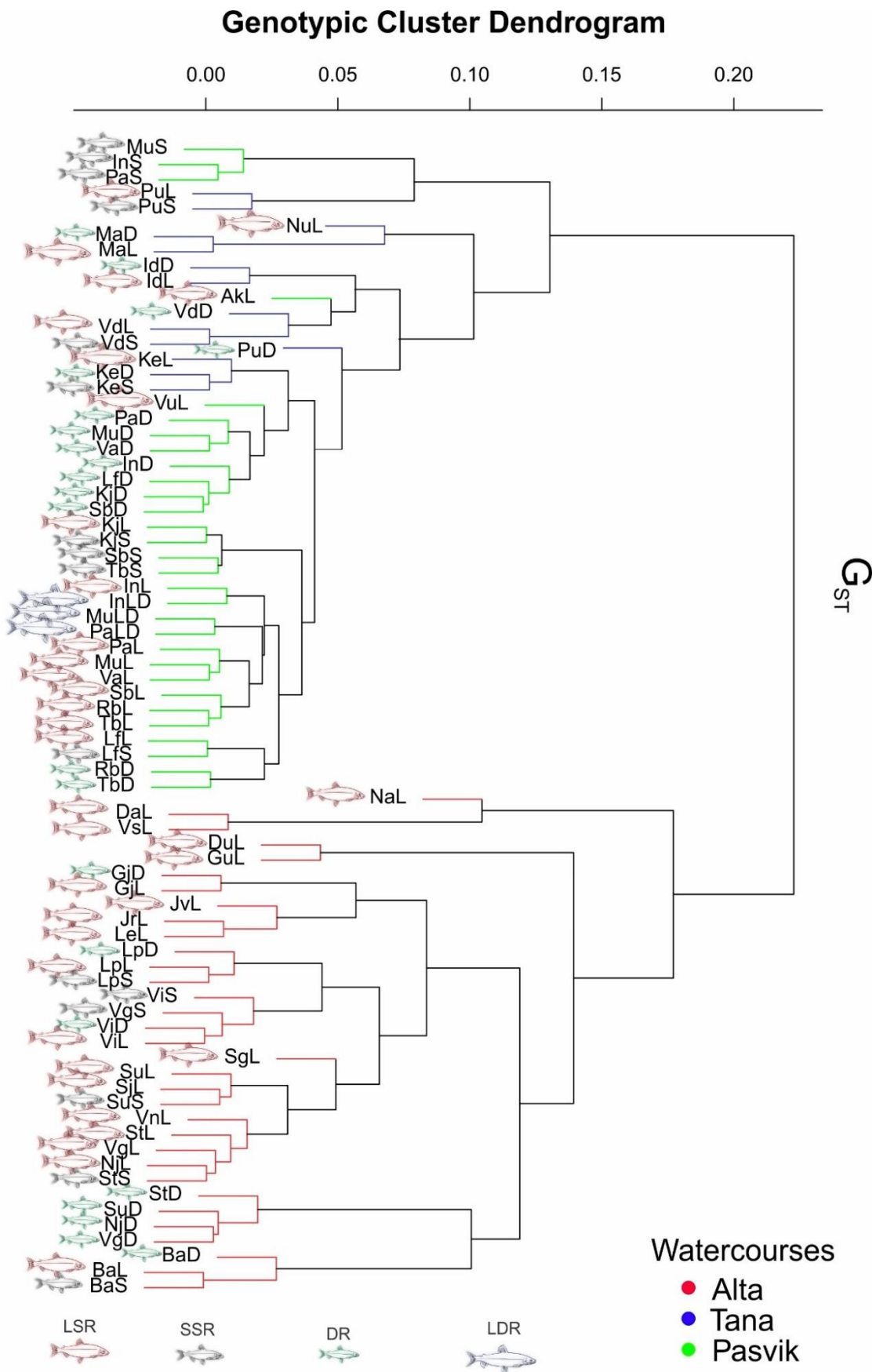


Figure 5. Dendrogram illustration of the pairwise G_{ST} between morphs in study lakes. For population abbreviations, see **Paper**

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 (Svärdson, 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

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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 km²) 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 ²)	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 ^a	40 ^a	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.
^aIn Inarjävri, sampling was confined to a single 32 km² 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 μ^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 θ^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 θ^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 θ^p was then used as a prior for Driftsel to estimate the posterior distributions of other parameters and to refine the estimate of θ^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 θ^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 θ^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 ± 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 ± 3.0), LSR whitefish intermediate (25.6 ± 4.7), and SSR whitefish the lowest (20.7 ± 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 ± 3.4 , Alta: 37.4 ± 3.5) compared to LSR (Tana: 23.4 ± 3.1 , Alta: 25.7 ± 2.5) and SSR whitefish (Tana: 22.7 ± 2.1 , Alta: 23.4 ± 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 Vuolgamasjavri, 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 ± 5.2 cm [*SD*]) than the mean total length of LSR (21.6 ± 6.5 cm) and SSR (21.3 ± 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 ± 2.7 cm) than LSR (19.4 ± 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 ± 4.7 cm) were smaller in size than the other morphs (LSR: 22.2 ± 6.7 cm, SSR: 22.9 ± 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 ± 2.5 cm; LSR: 22.9 ± 5.7 cm; SSR: 22.2 ± 4.8 cm) in any of the three lakes.

3.2 | Population-to-population coancestry matrix

The population-level coancestry coefficient matrix θ^P illustrates the relatedness between populations (Table S2). Mean estimates of diagonal elements in the coancestry matrix (θ_{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 (θ_{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 ≤ 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.

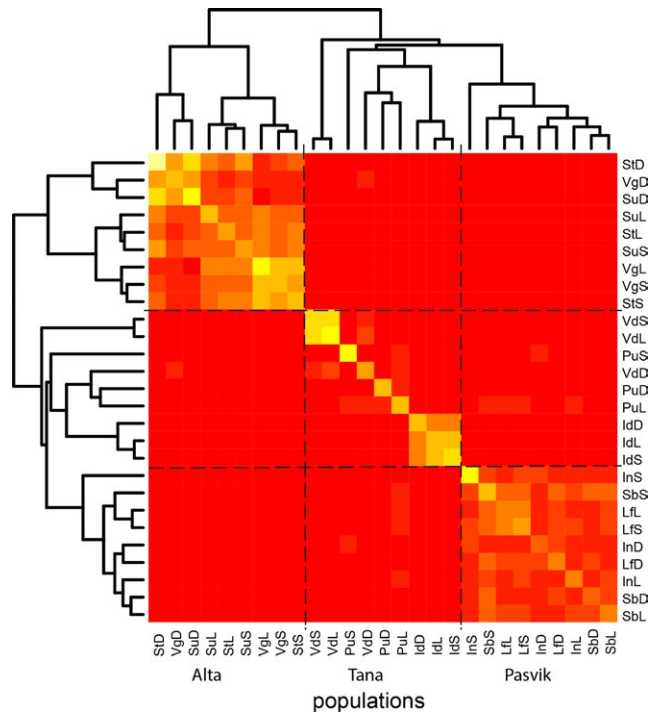


FIGURE 2 Heat map illustration of the coancestry matrix θ^P . Watercourses are separated with hatched lines. The lighter the color is, the more the populations resemble each other. Diagonal elements of matrix θ_{ii}^P represent random genetic drift. Dendrogram on the side illustrates the structure and 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 ± 5.6 cm [SD]) than females (mean 20.8 ± 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 ± 5.4 cm) than mature fish (mean 21.5 ± 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

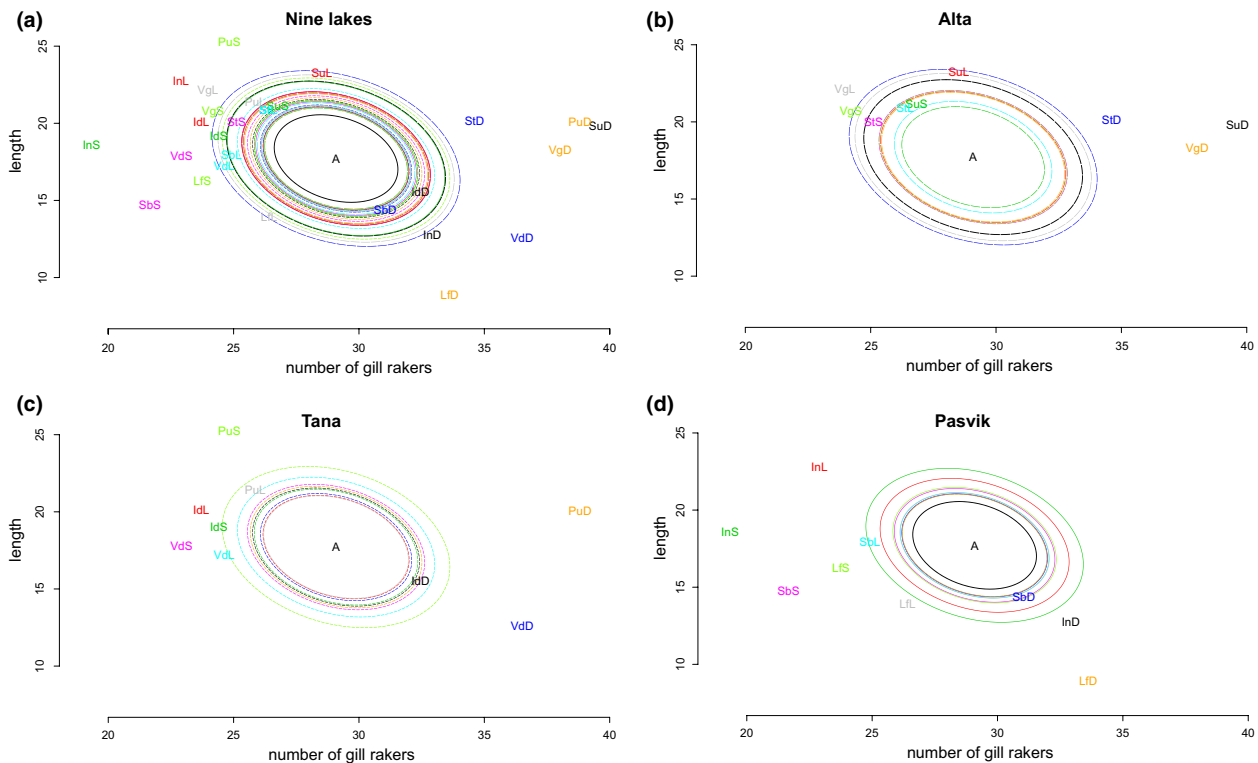


FIGURE 3 Observed divergence of phenotypic traits in all study lakes. Each ellipse represents the drift distance for the population of same color. Observed divergence in study lakes and populations (see abbreviations from Table 2). The position of the population codes represents population-specific level 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,

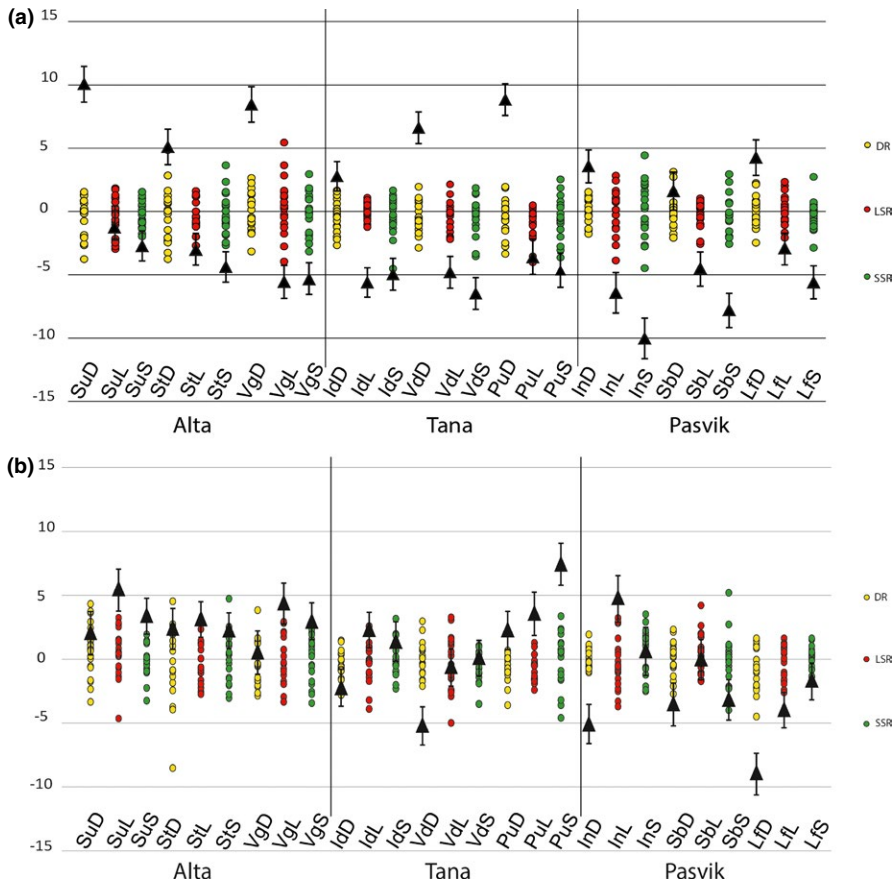


FIGURE 4 Population means in gill raker (a) and total length (b) traits. Triangular dots with error bars represent population means from the real data with 95% credibility interval. Small dots represent the amount of variation expected under drift-based divergence. Zero level is the ancestral mean. In 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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1 **(NON)PARALLEL PATTERNS OF STANDING GENETIC VARIATION AND**
2 **PHENOTYPIC DIVERSITY IN A SPECIATION CONTINUUM OF EUROPEAN**
3 **WHITEFISH IN NORTHERN FENNOSCANDIA**

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15 **Abstract**

16 Adaptive radiation is the evolution of ecological and phenotypical diversity that arises via
17 ecological opportunity and niche segregation. While the processes are increasingly studied in
18 single locations, we are generally lacking a landscape level approach to adaptive radiation that
19 takes into account colonization history and available genetic material, i.e. the standing genetic
20 variation that is crucial for natural selection to operate. In this respect, extensive adaptive
21 radiations of European whitefish (*Coregonus lavaretus* (L.)) in northern Fennoscandian lakes
22 provides a unique opportunity to assess the role of colonization history and standing genetic
23 variation on genotypic and phenotypic divergence at landscape level. We used a total of 36
24 lakes with 77 populations (total n=3430 ind) varying from single (n=14), two (n=6), three (n=13)

25 and four (n=3) sympatric morphs distributed on three neighbouring watercourses with known
26 differences in postglacial age for analyses of neutral genetic markers (19 microsatellite loci)
27 and phenotypic traits (gill raker count, body size, condition). We found that whitefish colonized
28 the watercourses from East (12000 years bp) to West (8700 year bp), with a resulting halving
29 of the standing genetic variation, likely due to colonization bottlenecks. While the amount of
30 morphs and their phenotypic divergence decreased with standing genetic variation, we found
31 genetically divergent morphs in each watercourse. Furthermore, genetic divergence between
32 the sympatric eco-morphs correlated with phenotypic divergence. Our results show that the
33 phenotypic variation in European whitefish do not predominantly reflect the amount of standing
34 genetic variation, despite clear genetic differences between watercourses. Therefore the
35 whitefish populations across northern Fennoscandia represent an example of (non)parallel
36 evolution, with parallel phenotypic and genotypic patterns lakewise, and in part non-parallel
37 between watercourses.

38 **Introduction**

39 Ecological opportunity enables exploration of new niches by providing a variety of distinctive
40 or underutilized resources (Schluter, 2000), which may lead to ecologically and phenotypically
41 diverged populations via adaptive radiation (Yoder et al., 2010). Plastic responses to
42 environmental differences may enable rapid adaptation in fluctuating environments, whereas
43 additive genetic changes may require longer time to accumulate in a population (Fierst, 2011).
44 However, both plastic and additive genetic responses are heritable and the evolvability of
45 expanding populations are anchored in the available amount of standing genetic variation
46 (Pfennig and Pfennig, 2010, Scheiner and Holt, 2012, Huang, 2015, but see also Sentis et al.,
47 2018). Exploration of new niches and the evolution of phenotype-environment correlations are
48 therefore assumed to be directly coupled with the available gene combinations, but to what
49 extent is still debated (Agashe and Bolnick, 2010). African cichlids is one of the most diverse
50 and well-known examples of adaptive radiation (reviewed in Salzburger, 2018). Previous
51 studies of cichlid radiations have revealed that the level of genetic divergence often is more

52 related to the geographical distance between populations than to the selective environments,
53 suggesting that phenotypic divergence can occur despite high gene flow, and that
54 morphological diversification is not necessarily accompanied by strong genetic differentiation
55 (Crispo and Chapman, 2008, Duftner et al., 2006). Pre-existing standing genetic variation
56 (SGV) has played an important role in this divergence process, where introgression and
57 recombination of genetic variation has presumably been major factors creating “new” genetic
58 material that selection can utilise (Salzburger, 2018).

59 Post-glacial lakes in northern Fennoscandia originates from the previous deglaciation,
60 when the Fennoscandian ice-sheet retreated from the northwestern Russia towards the west,
61 forming the three main contemporary watercourses, Pasvik, Tana and Alta approximately 15-
62 8.5 kyrs ago (Sollid et al., 1973, Andersen and Borns, 1994, Kujansuu et al., 1998, Mangerud
63 et al., 2004, Svendsen et al., 2004, Stokes et al., 2014, Stroeven et al., 2016). This deglaciation
64 process, where the ice-sheet rested in the western Alta region, took around 5000 years,
65 making the lakes in the Alta watercourse younger than lakes in the Tana and Pasvik
66 watercourses. It is assumed that whitefish followed the retreating glacial ice via the glacial
67 rivers and subsequently colonized the lakes when they became ecological available (Østbye
68 et al., 2005, Præbel et al., 2013a, Præbel et al. in prep). The many (>100) contemporary lakes
69 in these watercourses represent replicated environments that provides similar ecological
70 opportunities (Siwertsson et al., 2010), and similar potential for adaptive radiation. Because
71 of the colonisation history, whitefish occupying the Alta watercourse is considered to be about
72 1000 whitefish generation younger than whitefish populations in the older Tana and Pasvik
73 watercourses (Præbel et al., 2013a). Whitefish in this region originate from a single
74 monophyletic clade that, via ecological opportunity, subsequently radiated into distinct eco-
75 morphs (Østbye et al., 2005, Østbye et al., 2006, Præbel et al., 2013a).

76 Bolnick et al. (2018) introduced the term (non)parallel evolution to describe the
77 continuum from parallel to non-parallel evolution that is formed by the distribution of outcomes
78 across populations and traits. The postglacial lakes in northern Fennoscandia offer an

79 excellent avenue to study the extent of (non)parallel evolution with large spatial scale and
80 temporal transect originating from the colonization history of the area. This unique model
81 system, with both mono- and polymorphic whitefish populations, represents a speciation
82 continuum at the landscape level, where the most divergent polymorphic populations are found
83 in the presumably oldest Pasvik watercourse (Siwertsson et al., 2010). These polymorphic
84 populations are located close to the main stem of the river, where some of the lakes harbor up
85 to four distinct eco-morphs. Locally adapted polymorphic populations are common in
86 postglacial fishes. Arctic charr (*Salvelinus alpinus*) is a classical example of the vast amount
87 of phenotypic variation that can be found within fish species in postglacial lakes (Jonsson and
88 Jonsson, 2001, Recknagel et al., 2017), where divergence is often related to the trophic
89 behaviour (Klemetsen et al., 2006). Correspondingly in European whitefish, where natural
90 selection has shaped phenotypic characters in eco-morphs, the phenotypically divergent
91 populations are separated based on the trophic traits (e.g. number of gill rakers, head, and
92 body morphology) (Kahilainen and Østbye, 2006, Præbel et al., 2013a, Häkli et al., 2018). The
93 number of gill rakers is a heritable trait with strong correlation between parents and progeny
94 (Svärdson, 1952, Svärdson, 1979). Morphological differences in gill rakers affect the feeding
95 efficiently (Sanderson et al., 2001, Roesch et al., 2013) and have shown to be correlated with
96 habitat choice and/or prey selectivity in a range of fish species, such as e.g. sticklebacks
97 (Bolnick et al., 2009) and cichlids (Muschick et al., 2014). High number of gill rakers is
98 facilitating filter feeding on small zooplankton by increasing retention capability of zooplankton
99 prey (Roesch et al., 2013). Consequently, whitefish individuals can be sorted into different eco-
100 morphs, with the DR whitefish typically having a high number of gill rakers feeding zooplankton
101 in the pelagic zone, whereas more sparsely numbered gill rakers are typical for eco-morphs
102 feeding on benthic macroinvertebrates in the littoral (LSR whitefish) or in the profundal (SSR
103 whitefish) zone (Amundsen et al., 2004, Harrod et al., 2010, Kahilainen et al., 2011). In some
104 lakes in the oldest Pasvik watercourse, also large bodied densely rakered (LDR) whitefish
105 occurs feeding on zooplankton and surface insects in the interface of pelagic and littoral
106 habitats (Couton, 2012). All four morphs differ in their trophic ecology, morphology and life-

107 history traits making them important components in food webs of subarctic lakes (Kahilainen
108 et al., 2014, Kahilainen et al., 2017, Thomas et al., 2017, Thomas et al., 2019). Most of the
109 sampled monomorphic whitefish populations (LSR eco-morph) in our dataset are located in
110 the younger Alta watercourse, whereas most of the polymorphic populations are found in the
111 Pasvik watercourse (Siwertsson et al., 2010). LSR whitefish is considered as the ancestral
112 type from which the other eco-morphs have diverged (Østbye et al., 2005), and appears to be
113 the only eco-morph that is found in allopatry (Siwertsson et al., 2010). During the whitefish
114 colonization history when migrating from the Pasvik watercourse towards the Alta watercourse,
115 there were presumably a number of bottlenecks allowing just a subset of the original population
116 to continue further and colonized new areas. Suggestively, this diminishing front of colonizing
117 individuals may have majorly reduced the genetic variation in the Alta watercourse compared
118 to the ancestral Pasvik watercourse (Østbye et al., 2006, Häkli et al., 2018, Præbel
119 (unpublished)).

120 Here, we used this continuum of ongoing speciation to explore the distribution of
121 alternative levels of standing genetic variation across a landscape-wide gradient, and how it is
122 reflected in the contemporary phenotypic divergence. We predicted that the level of standing
123 genetic variation differs between watercourses, reflecting the distance from glacial refuge,
124 and expected to observe (non)parallel phenotypic differentiation based on the alternative levels
125 of standing genetic variation. For this purpose we explore adaptive phenotypic traits and key
126 life history traits of four different whitefish eco-morphs and contrast these with multilocus
127 genotypic data. The main objective was to study the correlation between phenotypic and
128 genotypic variation, and to elucidate how the evolution of morphotypes and adaptive
129 phenotypic traits have proceeded under alternative levels of standing genetic variation in an
130 ongoing adaptive radiation at a landscape-wide scale.

131

132 **Material and methods**

133 European whitefish were collected from 77 populations in 36 lakes across three watercourses
134 in northern Fennoscandia (Figure 1). A total of 19 study lakes were located in the Alta
135 watercourse, six in the Tana watercourse and 11 in the Pasvik watercourse. Here, 14 of the
136 study lakes harboured monomorphic populations, six lakes dimorphic population, 13 lakes
137 trimorphic populations and three lakes tetramorphic populations (Table 1). Fish were sampled
138 from littoral, pelagic and (when present; >10 m; <1% of light at surface) profundal habitats
139 using benthic and pelagic gill net series as described by Siwertsson et al. (2010). Each
140 individual was assigned to eco-morph in the field based on their phenotype as revealed from
141 gill raker and head morphology (Kahilainen and Østbye, 2006). We measured the total length
142 and weight, and counted the number of gill rakers from first left branchial arch. The age of each
143 individual was estimated by counting the annual rings from clear or burn and cracked otoliths.
144 A fin clip for genetic analysis was also obtained. Here, we used variation at 19 microsatellite
145 loci as a proxy of standing genetic variation. Number of gill rakers were counted on the first left
146 branchial arch under a microscope to verify eco-morph assignment. Here, the lowest amount
147 of very short and widely spaced gillrakers (typically <21), strongly subterminal mouth, robust
148 head and large eye positioned at dorsal part of head was typical for profundal benthivorous
149 SSR whitefish, whereas larger number of short gillrakers (typically <30) and smaller eye size
150 characterize littoral benthivorous LSR whitefish (Kahilainen and Østbye, 2006, Siwertsson et
151 al., 2010, Kahilainen et al., 2011). LDR whitefish is a large sized morph with large number of
152 long and densely spaced gillrakers (typically 30-38) use near surface habitats at pelagic
153 feeding on insects and zooplankton, while DR whitefish is small sized and dark colored pelagic
154 zooplanktivore with high number (typically 32-42) of fine and long gillrakers (Kahilainen et al.,
155 2014). Although the substantially diverged SSR and LDR whitefish eco-morphs are present
156 some of the lakes in older Pasvik watercourse, and SSR whitefish also in some lakes of Tana
157 watercourse, previous studies have revealed more subtle divergence between the littoral and
158 profundal whitefish in some lakes of the younger Alta watercourse (Siwertsson et al., 2013b).

159 Therefore, whitefish caught in the profundal habitat were coded as an SSR whitefish also in
160 the Alta watercourse, unless the fish was identified as a DR whitefish.

161 **Microsatellite DNA/Genotyping**

162 Genomic DNA was extracted from fin clips using E-Z96 Tissue DNA Kit (OMEGA Bio-tek)
163 following the manufacturer's protocol. A total of 19 microsatellite loci were amplified in four
164 polymerase chain reaction (PCR) multiplexes using forward-labelled primers as described in
165 the protocol of Præbel et al. (2013b). Replicate (5-9 %) and blind (4 %) samples were included
166 in all PCR's to confirm consistency of scoring and the absence of cross contamination. The
167 PCR products were separated on an ABI 3130 XL Automated Genetic Analyzer (Applied
168 Biosystems) using GENESCAN LIZ-500 (Applied Biosystems) as an internal size standard.
169 The binning and scoring were performed in GENEMAPPER 3.7 (Applied Biosystems) and
170 verified manually. To detect possible null alleles and genotyping errors, microsatellite data was
171 tested with the MICRO-CHECKER 2.2.3 software (Van Oosterhout et al., 2004). Deviations
172 from linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) were tested per locus
173 over all populations using exact tests (Guo and Thompson, 1992) as implemented in GenePop
174 4.0 (Rousset, 2007). The pairwise comparisons were corrected for multiple comparisons using
175 Sequential Bonferroni corrections (BFC) following Rice (1989).

176 **Estimating standing genetic variation**

177 The number of alleles in a population may be affected by random drift as rare alleles are likely
178 to be lost in bottlenecks, whereas expected heterozygosity is not that sensitive to possible
179 bottlenecks (Allendorf, 1986). We used the R statistical computing programme (R Core Team,
180 2017) to calculate four genetic traits to illustrate genetic variation among individuals and
181 populations: number of alleles (N_a), expected- (H_e) and observed heterozygosity (H_o) per
182 population, and sample-size corrected standardised allelic richness (A_r). H_e and H_o was
183 estimated using *genhet* R-function (Coulon, 2010), and A_r using R package *PopGenReport*
184 (Adamack and Gruber, 2014). In addition, two measures of population genetic differentiation,

185 Nei's G_{ST} and Jost's D were calculated for all population pairs with *MMOD* R-package (Nei,
186 1973, Nei and Chesser, 1983, Jost, 2008, Winter, 2012), as markers where G_{ST}
187 underestimates divergence should have significantly elevated values of D . G_{ST} is the multi loci
188 analog to the F_{ST} fixation index, that was originally developed for biallelic markers. Multivariate
189 analysis of genotypic data was performed with *adegenet* (Jombart, 2008) and *ape* (Paradis et
190 al., 2004) packages in R. *Adegenet* uses allelic frequencies of genotypes and counts of alleles
191 per population when performing multivariate analyses. Missing data (1.2%) was replaced with
192 a mean allele frequency value, which is the default setting in *adegenet*. We implemented
193 principal component analysis (PCA) to investigate the variability in genotypic data, and used
194 the calculated PC's to represent the genetic data in a redundancy analysis, which summarise
195 the variation in response variables that can be explained by explanatory variables (see below).

196 **Estimating phenotypic variation**

197 We used three phenotypic traits to describe variation between and among eco-morphs; gill
198 raker number, standardised length (as a deviation from the mean length per age class, all
199 populations combined, where age classes 11-12, and 13+ were pooled together due to
200 decreasing number of older individuals) and Fulton's condition factor (K). K can be used to
201 describe in how good shape and how heavy fish are compared to their length (Ricker, 1975,
202 Barnham and Baxter, 1998), and was used as a proxy for fitness herein. As fish length and
203 weight are polygenic traits with small to moderate heritability in salmonids (Gunnes and
204 Gjedrem, 1978, Refstie and Steine, 1978, Gjerde and Gjedrem, 1984, Gjerde and Schaeffer,
205 1989), we note that environmental factors, such as time of the year or productivity of the lake,
206 may have larger effect on them than on e.g. the number of gill rakers. Multivariate analysis of
207 variance (MANOVA) was used to assess if grouping to watercourse, lake, and eco-morph,
208 respectively, explained a statistically significant amount of variance in the phenotypic traits. In
209 addition, we used *ade4* (Dray and Dufour, 2007) and *Vegan* (Oksanen et al., 2015) R packages
210 for phenotypic PCA analyses and data visualization. We excluded 10 individuals from further

211 analysis because of unrealistic high or low K values, probably originating from writing mistakes
212 on field notes.

213 **Association between phenotypic and genetic variation**

214 We calculated Mahalanobis distance between the polymorphic populations using the three
215 phenotypic traits: number of gill rakers, standardised length, and Fulton's condition factor K.
216 Mahalanobis distance computes pairwise comparison of group means using the correlation
217 between variables, i.e. it takes into account different variances and correlations between
218 variables (McFerrin, 2013). This phenotypic distance was then compared (as proportion of
219 maximum observed distance) with the genotypic G_{ST} and D matrixes to reveal if the genetic
220 differentiation was accompanied with phenotypic differences. We performed two different
221 comparisons: a landscape-wise to assess how phenotypic and genetic differences changes
222 within eco-morphs across the lakes in the three watercourses, and lake-wise to investigate the
223 level of divergence within each lake. Morph-wise comparisons across the landscape were
224 made against the ecomorph in question in lake Skrukkebukta, which was the north-
225 easternmost lake in our dataset. The only exception was the three LDR populations, that were
226 compared against DR, as LDR is not present in Skrukkebukta. Pairwise comparisons between
227 eco-morphs were performed separately in each lake. Furthermore, to test whether the
228 differences on phenotypic traits align with the standing genetic variation (allele frequencies),
229 we performed a redundancy analysis (RDA), which summarise the variation in response
230 variables that can be explained by the explanatory variables using vegan R package (Oksanen
231 et al., 2015). Here, the first 10 PCA axis calculated from the 19 microsatellite loci represented
232 the genetic variation in the dataset. Statistical significance of RDA was verified with
233 permutation test. In addition, we tested if the proportion of heterozygous loci (PHt) correlated
234 with condition factor to assess possible fitness advantages as microsatellite marker
235 heterozygosity has shown to correlate with fitness traits (Bierne et al., 1998, Coulson et al.,
236 1998, Coltman et al., 1999). This individual heterozygosity estimate was calculated with

237 GENHET R function (Coulon, 2010). As the number of alleles differed between watercourses,
238 Pearson's correlation analysis was calculated separately for each of the three watercourses.

239 **Results**

240 **Genetic variation**

241 Number of alleles at each microsatellite locus ranged from 5 to 39 across all lakes and eco-
242 morphs, where the total number of detected alleles in 77 populations across 19 loci was 348
243 (Table S1). Deviations from Hardy-Weinberg equilibrium (HWE) were indicated in 43 out of
244 1463 tests (2.94%) after sequential Bonferroni corrections, which is less than expected by
245 chance (5%). Only 30 (0.23%) out of 13167 loci comparisons were significant for linkage
246 disequilibrium (LD) after Bonferroni corrections. Sample size had a small, but statistically
247 nonsignificant effect on the number of alleles in the populations (adjusted $R^2 = 0.021$, p-value
248 = 0.11), and became antagonistic when only considering the monomorphic populations, i.e.
249 populations with larger sample size had fewer alleles (Figure S1 and S2). Average observed
250 heterozygosity varied from 0.27 to 0.66, and average allelic richness from 2.17 to 6.74. The
251 expected heterozygosity was larger than the observed heterozygosity, with the mean of the
252 differences being 0.016. Both allelic richness and heterozygosity decreased when comparing
253 the Tana with the Pasvik watercourse, and were lowest in the Alta watercourse ($p < 0.001$)
254 (Figure 1).

255 Visualization of the principal component analysis of microsatellite data revealed two
256 clearly separated clusters: Alta watercourse, and Pasvik-Tana watercourses (Figure 2 and 3).
257 The first PC axis divided especially Alta further from the older watercourses, explaining 3.0%
258 of the total variation ($p < 0.001$ for all comparisons). The second PC axis represented the
259 differences between the populations in the Tana watercourse compared to the Pasvik and Alta
260 watercourses, and explained 0.9% of the total variation ($p < 0.001$, Pasvik vs Alta n.s.)(Figure
261 2 and 3). This division was more distinct when analysing only monomorphic populations
262 (Figure 3). Pairwise G_{ST} estimates between eco-morphs ranged from 0 to 0.223 with an

263 average G_{ST} of 0.082, and the differentiation increased with the distance to the north-
264 easternmost lake Skrukkebukta. In other words, geographic distance correlated with genetic
265 distance to the direction of hypothesized colonization route from east towards west (Figure S3).

266 **Phenotypic variation**

267 Gill raker number varied from 14 to 47, fish length from 6.1 to 50.7 cm with deviation from
268 mean length at year class from -2.41 to 4.86 sd, and condition factor K from 0.52 to 1.54 in the
269 36 study lakes (Figure 4, Table S2). As expected, DR and LDR whitefish had the highest, LSR
270 the intermediate, and SSR the lowest number of gill rakers, whereas condition factor and length
271 at age varied more between watercourses. The multivariate analysis of variance revealed
272 statistically significant ($P < 0.001$) differences in phenotypic traits of fish between grouping
273 variables of lake, watercourse and eco-morph classification, i.e. there were watercourse- and
274 lake-specific differences between eco-morphs. In general, fish in the Alta watercourse were
275 larger (mean length at age for DR, LSR and SSR: -0.18, 0.61, 0.05, respectively, $p < 0.001$) and
276 in better shape (mean K for DR, LSR and SSR: 1.03, 1.06, 1.02, respectively, $p < 0.001$)
277 compared to the other two watercourses (mean length at age: -0.50, 0.12, -0.57 and K: 0.89,
278 0.91, 0.81 in Tana for DR, LSR and SSR, and mean length at age: -0.73, 0.91, 0.10, -0.99 and
279 K: 0.86, 0.80, 0.90, 0.90 in Pasvik for DR, LDR, LSR and SSR, respectively) (Figure 4, Table
280 S2). The first two axes in the PCA of the phenotypic traits explained 74.9% of the total variance,
281 and illustrated the subtle differences between populations in Alta and the older Pasvik and
282 Tana watercourses. However, rather than clustering the eco-morphs according to their original
283 watercourse (Figure 5 and S5), the PCA of the phenotypic traits clustered each eco-morphs
284 together across watercourses (Figure 5), supporting parallel phenotypic evolution across a
285 post-glacial landscape. The phenotypic differences among eco-morphs between the three
286 watercourses changed gradually from the Pasvik to the Alta populations. In other words, there
287 were subtle phenotypic differences both between and within watercourses. Thus, the
288 phenotypic PCA represented a continuum of populations where the x-axis was associated with
289 fish size and the y-axis with the number of gill rakers.

290 **Association between phenotypic and genetic variation**

291 Landscape wide comparison of Mahalanobis phenotypic distance and G_{ST} revealed a weak,
292 but statistically significant (overall $R^2 = 0.086$, $P = 0.023$) trend between phenotypic and
293 genotypic distances, i.e. an increasing genetic distance between populations within eco-
294 morphs was also associated with a slight increase in phenotypic distance between populations
295 (Figure 6). However, when looking at each of the eco-morphs separately, these trends become
296 statistically non-significant. The lake-wise comparisons between eco-morphs in their
297 respective lakes (Figure 7) revealed stronger positive trend between phenotypic and genetic
298 distances (overall $R^2 = 0.34$, $P < 0.001$) and all eco-morph pair comparisons were statistically
299 significant (DR-LSR: $R^2 = 0.21$, $P = 0.032$, DR-SSR: $R^2 = 0.53$, $P = 0.001$, LSR-SSR: $R^2 =$
300 0.65 , $P < 0.001$). The association between phenotypic traits and underlying genetic variation
301 was estimated using redundancy analysis, where the first 10 axis from the genotypic PCA was
302 used to represent the genotypic data. The multivariate linear relationship between three
303 phenotypic traits and genetic variables was weak, but statistically significant ($P < 0.001$). The
304 redundancy analysis explained 11.7% of the total variation, where the first RDA1 axis
305 explained 7.9% and second RDA2 axis 1.4% of the variation (Figure 8). Both Fulton's condition
306 factor K and length of the fish increased towards the populations in the Alta watercourse, were
307 the homozygosity per locus was greatest and the number of alleles smallest compared to the
308 populations in the Tana and Pasvik watercourses. Analysis of the monomorphic populations
309 revealed a similar pattern, with the genetic variation being smaller in lakes in the Alta
310 watercourse, where the populations were characterised by larger size at age and condition
311 factor. We observed a statistically significant ($p=0.033$), but weak ($r=0.056$) positive correlation
312 between heterozygosity and condition factor in the Alta watercourse (Figure S4). In the Tana
313 watercourse the correlation was negative ($p < 0.001$, $r = -0.200$), and in the Pasvik watercourse
314 it was close to zero and statistically non-significant ($p=0.622$, $r=0.014$).

315

316 **Discussion**

317 Here we demonstrated how genetic divergence between the sympatric eco-morphs correlated
318 with phenotypic divergence, but despite clear genetic differences between watercourses, the
319 phenotypic variation within European whitefish eco-morphs do not necessarily follow the same
320 trajectory. We investigated how one of the basic components of evolutionary diversification,
321 the level of standing genetic variation (e.g. Barrett and Schluter, 2008), reflected adaptive
322 phenotypic variation in European whitefish eco-morphs at a landscape wide scale. We
323 observed both parallel and non-parallel phenotypic and genetic differentiation across the study
324 populations, despite sympatric diversification and parallel evolution of eco-morphs being the
325 accepted mechanisms of adaptive radiation within this landscape of lakes (Østbye et al., 2006,
326 Siwertsson et al., 2010, Kahilainen et al., 2011, Præbel et al., 2013a, Siwertsson et al., 2013b).
327 In the oldest watercourse, Pasvik, phenotypic diversification has proceeded the most as four
328 eco-morphs have evolved in some lakes, whereas the three main eco-morphs also were
329 detected in each of the two younger watercourses. However, we found that despite the drastic
330 decrease in SGV from the older Pasvik watercourse towards the youngest Alta watercourse,
331 the level of phenotypic differentiation varied across the studied landscape, and thus
332 represented a gradient of phenotypic variation from small to large phenotypic differences.
333 Variation of phenotypic traits explained only a small, but statistically significant, proportion of
334 the genetic variation. The repeated parallel evolution of ecotypes is considered to be the
335 product of natural selection and adaptation (Bolnick et al., 2018), but some degree of non-
336 parallelism is also commonly found in whitefish populations, where phenotypic responses to
337 ecological variation may depend on genetic background of populations (Evans et al., 2013,
338 Siwertsson et al., 2013a). Here we will discuss these results in light of historical contingency,
339 ecological variables and ecological speciation.

340 Our findings revealed that both the number of alleles and the observed heterozygosity
341 were almost half in the Alta watercourse compared to the Pasvik watercourse. Populations'
342 adaptive capability is closely linked to the available standing genetic variation (Barrett and

343 Schluter, 2008, Ørsted et al., 2019), where the available allelic variants and/or epigenetic
344 genomic alterations may have profound effect on a species ability to explore and adapt to new
345 ecological niches and habitats (Barrett and Schluter, 2008, Anderson, 2012, Paaby and
346 Rockman, 2014). During the last glaciation, European whitefish presumably used lake Onega
347 as a refuge (Østbye et al., 2005), and during the deglaciation whitefish utilized the west to east
348 flowing melting rivers to recolonize the landscape. Postglacial range expansions are expected
349 to be affected by founder effects due to colonization history, leading to loss of genetic diversity
350 and increasing homozygosity (Hewitt, 1996). Indeed, repeated bottlenecks have presumably
351 left their marks on the whitefish populations with decreasing amount of genetic variation from
352 the Pasvik to the Alta watercourse. Our results confirmed this pattern as allelic richness in the
353 Alta watercourse was just a subset of the variation in the Pasvik watercourse, where also
354 heterozygosity was greater than in the lakes in the Alta watercourse. Similar consequences,
355 with increased homozygosity and spatial assortment of genotypes with frequent geographic
356 subdivisions, have been shown in several species after postglacial colonization (e.g. Hewitt,
357 1999, Adams et al., 2006). Our results thus suggest that the decrease in SGV may be a
358 signature of repeated bottlenecks from the post glacial recolonisation, where only fractions of
359 the original founder population migrated and colonized lakes as they appeared and became
360 ecological active.

361 It should be emphasized that we used microsatellite loci as a proxy for standing genetic
362 variation to allow for exploration at the landscape-wide pattern of variation. Microsatellite loci
363 may not accurately reflect the genetic variation at additive loci and loci contributing to the
364 phenotype (Reed and Frankham, 2001, Moss et al., 2003), but Fischer et al. (2017) has shown
365 that F_{ST} comparisons based on microsatellites and genome-wide SNP's are correlated, and
366 that microsatellite allelic richness (A_r) served as a proxy for SNP diversity. Thus, microsatellites
367 may still represent a view into the standing genetic variation of populations due to their
368 genome-wide origin. In addition, our results from microsatellite data are in consensus with
369 genome wide SNP data from RAD-sequencing (Præbel et al. in prep). Therefore we trust that

370 neutral variation is a good indicator for the standing genetic variation in these lakes. Our
371 results also showed that whitefish have approximately similar standing genetic variation within
372 the watercourses, suggesting that each of the watercourses was colonized at single events in
373 evolutionary time. For the west to east flowing Tana watercourse, whitefish populations in the
374 eastern lakes showed higher standing genetic variation than whitefish populations in the more
375 western lakes. This pattern of standing genetic variation across the landscape was also
376 supported from the genetic analyses where whitefish clustered genetically according to their
377 original watercourse. Despite the smaller amount of genetic variation in the Alta watercourse,
378 the three eco-morphs have also radiated there, although the phenotypic divergence among
379 them is more subtle compared to the Pasvik and Tana watercourses. Simulations has revealed
380 that niche founders may on average be more evolvable, which may provide a genotypic bias
381 towards evolvability as population grow up (Lehman and Stanley, 2013). Therefore the newly
382 established popoulation may on avarage be more evolvable than the population from which it
383 originates. If this holds true regardless of the smaller amount of genetic variation present in
384 Alta, remains unanswered.

385 The analyses across watercourses revealed a less evident landscape-wide pattern in
386 phenotypic trait variation compared to the genotypic pattern. Even though the phenotypic PCA
387 assigned whitefish in the Alta watercourse slightly further apart from the whitefish in the Pasvik
388 and Tana watercourses, the phenotypic clustering of the different eco-morphs formed a
389 continuum across the watercourses. In the Alta watercourse, fish were characterised by a
390 larger body size, as both length at age and K were larger compared to the fish in the older
391 Tana and Pasvik watercourses. As length and weight are polygenic traits with small to
392 moderate heritability (Gunnes and Gjedrem, 1978, Refstie and Steine, 1978, Gjerde and
393 Gjedrem, 1984, Gjerde and Schaeffer, 1989) it is expected that environmental factors, like prey
394 availability, competition, and predation have large effect on both length and fish size. Indeed,
395 increased competition and predation have shown to reduce the fitness and/or growth in
396 multiple fish species (Hazlerigg et al., 2012, Hall and Kingsford, 2016, Tadesse, 2018). It has

397 been suggested that predation pressure on DR whitefish is larger compared to the other eco-
398 morphs, as DR whitefish is the main prey for piscivorous fish such as pelagic brown trout
399 (Kahilainen and Lehtonen, 2003, Jensen et al., 2008). Obviously, predation induced mortality
400 is dependent on abundance of piscivorous fish. In the lakes in the Pasvik and Tana
401 watercourses, where the DR whitefish are substantially smaller in size than the other eco-
402 morphs, predator populations are abundant and diverse, which contrasts the Alta lakes where
403 the number of other fish species in general is lower. The larger amount of fish species may
404 also indicate increased density-dependent resource competition between and among fish
405 species in the older watercourses. Therefore, the differences in fish length and K for the DR
406 ecomorph between the Alta and Pasvik watercourses could also be driven by ecological
407 factors, such as resource competition and predation. To disentangle the relative role of
408 predation and SGV for the evolutionary trajectory of this speciation continuum, future studies
409 should focus on comparing lakes with and without predators among these systems.

410 Our study revealed a weak correlation between phenotypic trait variation and standing
411 genetic variation. Linking standing genetic variation and phenotypic variation is not a straight
412 forward task in natural systems, as geological and glacial history, ancestral genomic standing
413 variation, time, and mode of speciation may all have impacted the contemporary variation and
414 architecture of traits (Hewitt, 1996, Butlin et al., 2008, Koizumi et al., 2012, Morris et al., 2018).
415 The ultimate goal for this exercise is to identify the genetic basis of phenotypic traits evolving
416 for adaptation to new environments and ecological niches. There are only a few examples of
417 such findings from a few species, like the genetic basis of phenotypic diversity in sticklebacks
418 (Peichel and Marques, 2017), wing patterning in butterflies (Kronforst and Papa, 2015), and
419 time at sea in salmon (Barson et al., 2015), despite a rapidly increasing use of genome wide
420 tools in evolutionary research. A part of this exercise is to understand how alternative levels of
421 standing genetic variation within the same species may produce similar or different phenotypic
422 variation. Hence, correlation between phenotypic trait variation and standing genetic variation
423 at large multi-system scales may provide important insights into which and how adaptive traits

424 evolve during incipient speciation. Herein, we found that variation in number of gill rakers were
425 more correlated with the high SGV populations in the older Pasvik and Tana watercourses,
426 whereas length and condition factor were more correlated with populations with lower SGV in
427 the younger Alta watercourse. We have previously shown that the number of gill rakers and fish
428 length are adaptive traits that are influenced by diversifying selection within lakes (Häkli et al.,
429 2018) and are important for the ongoing speciation process in these systems (Østbye et al.,
430 2006, Præbel et al., 2013a, Siwertsson et al., 2013b). From the present results, it appears that
431 the importance of these traits should be evaluated more thoroughly, as we cannot disentangle
432 whether the trait correlations are related to different levels of SGV or to different evolutionary
433 ages of the watercourses. However, theoretically, it must be assumed that the number of gill
434 rakers must be more important for the divergence in younger systems, where the reproductive
435 barriers are weak, compared to the older systems. This is because the number of gill rakes
436 are highly correlated with niche utilization (Kahilainen et al., 2011). A similar hypothesis may
437 be forwarded for the length and condition factor in the older systems, where size based
438 assortative mating and differences in life history traits may have enforced the reproductive
439 barriers between eco-morphs.

440 The occurrence of phenotypically and genetically differentiated populations in sympatry
441 has been documented as a widespread phenomenon in Northern European lakes (Taylor,
442 1999, Wilson et al., 2004, Klemetsen, 2010), and have been hypothesized to be a product of
443 incipient speciation driven by resource polymorphism (Skúlason et al., 2019). Arctic charr, the
444 world's northernmost freshwater fish, is a polymorphic fish species displaying sympatric
445 populations with varying amount of genotypic substructure and phenotypic differences
446 throughout its distributional range (Jonsson and Jonsson, 2001, Shikano et al., 2015, Jacobs
447 et al., 2018). European Whitefish in northern Fennoscandia follow a similar trajectory, where
448 eco-morphs show varying degrees of phenotypic and genotypic divergence in sympatry at a
449 large landscape scale. Thus, these systems represent a speciation continuum, where the most
450 phenotypically diverged populations, and most of the polymorphic lakes, are found in the

451 Pasvik watercourse (Siwertsson et al., 2010), near the glacial refugium (Østbye et al., 2005).
452 The number of polymorphic lakes decreases over the landscape towards the West, and the
453 Alta watercourse harbour the fewest polymorphic lakes. Differentiation between and among
454 populations can be evaluated at different levels, including polygenic phenotypic traits,
455 monogenic phenotypic traits, and genetic markers (both adaptive and neutral). From an
456 evolutionary perspective, investigating the relationship between phenotypic differentiation and
457 genotypic diversity is important for understanding the evolutionary forces shaping the observed
458 geno-phenotypic differentiation. Notwithstanding, a meta-analysis of 71 species have shown
459 that the diversity at genetic markers do not necessarily reflect the quantitative phenotypic
460 diversity, when selection drives the phenotypic differentiation (Reed and Frankham, 2001). In
461 fishes, adaptive evolution rarely seems to involve the fixation of beneficial alleles. Most often
462 the adaptive traits are highly polygenic, and soft selective sweeps are expected to be involved
463 in the adaptations that seem to proceed by shifts in allele frequencies that are shared between
464 differentially adapted population (Bernatchez, 2016). In the present study, the landscape-wide
465 phenotypic divergence within eco-morphs was not as affected by the increasing geographic
466 distance as the genetic divergence. More specifically, we observed a triangular relationship
467 between the phenotypic and genetic distance when comparing eco-morphs across the three
468 watercourses, i.e; small genetic distances were mainly associated with low phenotypic
469 distances, whereas larger genetic distances corresponded to low to high phenotypic distances.
470 A similar pattern with non-linear relationships between genetic and phenotypic distances have
471 been observed in natural populations as well as in theoretical and experimental studies in other
472 taxa (Burstin and Charcosset, 1997, Mullen Lynne et al., 2009). Despite the increasing genetic
473 distances across the three watercourses (in each eco-morph), the phenotypic differences did
474 not followed the same pattern and there was only a small increase in phenotypic distance with
475 increasing geographical distance to the presumed ancestral population. This pinpoint the
476 independent evolution of morphs, as also observed in lake whitefish (*Coregonus clupeaformis*)
477 (Renaut et al., 2011). The phenotypic distance increased with the genetic distance when
478 comparing sympatric eco-morphs within each lake. Here large genetic distances were

479 associated with large phenotypic distances, whereas small genetic distances were associated
480 with both small and large phenotypic distances. This was most distinct when looking at the
481 DR-LSR comparisons. As gene flow and hybridisation are known to slow down the genetic
482 segregation, whereas RI reinforces it, the assumption that larger genetic differentiation is also
483 associated with more profound phenotypic differences in sympatric populations is justified. The
484 lower amount of genetic differentiation compared to phenotypic variation, that were observed
485 in some sympatric comparisons, may refer to directional selection or phenotypic plasticity (Ben
486 Cohen and Dor, 2018), whereas genetic variation without phenotypic differences, like observed
487 within eco-morph comparisons, may be the outcome of convergent evolution (Losos, 2011).
488 Therefore the observed phenotypic and genetic divergence in whitefish populations may be
489 results of non-parallel and convergent evolution between watercourses and parallel evolution
490 within each watercourse.

491 The genetic differences between eco-morphs were in general small, suggesting that
492 the reproductive barriers are yet to be reinforced (Bitz-Thorsen et al., 2019). Size-assortative
493 mating has suggested to be an important mechanism maintaining reproductive isolation
494 despite gene flow (Bhat et al., 2014). Size assortative mating may arise as whitefish eco-
495 morphs differ in size, and it has shown that phenotypic differences, rather than genetic
496 distance, promotes assortative mating between eco-morphs in adaptive radiation (Stelkens
497 and Seehausen, 2009). Indeed, size based assortative mating has been observed in some
498 salmonids, including whitefish (Svårdson, 1979, Foote, 1988). In our study lakes, the
499 distributions of phenotypic trait outcomes was also oftentimes overlapping between eco-
500 morphs, especially in the Alta watercourse, despite significant genetic differences. This
501 suggests that reproductive isolation mechanisms between morphs within lakes exists. As the
502 phenotypic differences between morph-pairs are small, it is unlikely that they promote
503 assortative mating. Simulations has shown that in case of divergent selection, adaptation from
504 SGV can facilitate the progress of speciation because pleiotropic effects of alternative alleles
505 change the major axes of phenotypic variation in hybrids and reduce their fitness in parental

506 habitats (Thompson et al., 2019). However, the fitness of hybrids and the parental eco-morphs
507 have yet to be studied in our systems. Additionally, studies have shown that prezygotic
508 isolations evolves well before postzygotic isolation in several fish species (Bolnick and Near,
509 2005, Stelkens et al., 2010). Indeed, a recent study in the Alta watercourse has highlighted
510 allochrony, i.e. differences in spawning time, as an important contributor for building up the
511 reproductive barriers and reinforcing assortative mating between eco-morphs (Bitz-Thorsen et
512 al., 2019). While such temporal differences in spawning time are typical for polymorphic
513 whitefish across the distribution (e.g. Svärdson, 1979, Johansson, 2017), the initial
514 development of divergence are less explored. From the studied watercourses, the spawning
515 times seems to be linked in divergence of whitefish eco-morphs resource use in lake habitats
516 having different prey availabilities and temperatures (Kahilainen et al., 2014). Littoral habitats
517 warm up early in the summer and provide abundant benthic invertebrate resources for fast
518 growing LSR whitefish, whereas summer temperatures are lowest in deepest profundal habitat
519 that has also the lowest prey availabilities, delaying SSR whitefish eco-morphs energy gain for
520 somatic and gonadosomatic growth (Kahilainen et al., 2014). In contrast to profundal, pelagic
521 resources are available in abundance only during short summer timeframe, and maturation of
522 pelagic morphs (DR and LDR whitefish) occurs earlier than littoral LSR whitefish and latest
523 spawning SSR whitefish in both Pasvik and Alta watercourses (Kahilainen et al., 2014, Bitz-
524 Thorsen et al., 2019). Such similarity in allochrony in many lakes in different watercourses
525 strongly suggest similar ecological and environmental conditions driving reproductive isolation
526 among the whitefish eco-morphs, and likely explains the observed genetic differentiation even
527 with subtle phenotypic differences.

528 Although (environmental) lake conditions seems relatively similar across the three
529 watercourses, there are some differences. For example, eastern lakes are larger,
530 deeper, more productive and situated at lower altitude suggesting higher temperatures
531 compared to western lakes (Siwertsson et al., 2010). Such conditions likely provide high
532 ecological opportunities for whitefish to diverge, and addition to historical contingency that only

533 subset of other freshwater fish species were able to colonize these lakes (Siwertsson et al.,
534 2010). Ecological opportunity has played an important role in several examples of ecological
535 speciation (Losos, 2010, Yoder et al., 2010) and also for incipient speciation in European
536 whitefish (Siwertsson et al., 2010, Kahilainen et al., 2011, Siwertsson et al., 2013b). Apart from
537 purely plastic responses to environmental differences, genetic variation has been thought to
538 set the frame for populations' evolvability. But the question of how much variation is needed,
539 still remains unanswered. We observed (non)parallel evolution across watercourses as eco-
540 morphs where phenotypically recognised in all watercourses, but the degree of phenotypic
541 differentiation among watercourses was not equal and was not strongly associated to the
542 standing genetic variation. The linear relationships between phenotypic and genetic variation
543 were moderate to weak, indicating that the phenotypic variation do not necessarily reflect the
544 underlying (neutral) genetic variation. Also, when comparing the phenotypic and genetic
545 distances within eco-morphs across the landscape, the large genetic distances were
546 associated both with large and small phenotypic distances, highlighting the (non)parallel nature
547 of the speciation continuum of European whitefish in northern Fennoscandia. In addition,
548 marker heterozygosity had positive correlation with condition factor only in the youngest
549 watercourse, suggesting watercourse specific differences across the study lakes. We therefore
550 conclude that the evolution of European whitefish eco-morphs is an intricate case of parallel
551 and non-parallel speciation of phenotypes, where the allelic variants underlying adaptive traits
552 may be present or new combinations have evented at the different levels of available standing
553 genetic variation.

554

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875

876 **Figures and tables**

877 **Figure legends**

878 **Figure 1.** Map of the study area, summary plot of allelic richness and observed heterozygosity
879 between the lakes in the three watercourses, and number of fish species in each lake. Lakes
880 in Alta, Tana and Pasvik watercourses are coded with red dots, blue triangles and yellow stars.
881 Contour lines represent the retreating ice. Violin plots of allelic richness and observed
882 heterozygosity displays the range of results, whereas the shape represent the (mirrored)
883 density plot, revealing where most of the results are located. Sampling sites are coded with
884 abbreviations of lake names: Ak = Aksujärvi, Ba = Bajasjavri, Da = Dátkojávri, Du =
885 Durbunjavri, Gj = Gædgejavri, Gu = Guorbajavri, Id = Iddjavri, In = Inarijärvi, Jr = Jårgajavri,
886 Jv = Jevdesjavri, Ke = Kevojärvi, Kj = Kuetsjarvi, Le = Læmbejavri, Lf = Langfjordvatn, Lp =
887 Lahpojavri, Ma = Mohkkejavri, Mu = Muddusjärvi, Na = Nakkitjavri, Nj = Njallajavri, Nu =
888 Nuorbejavri, Pa = Paadarjärvi, Pu = Pulmankijärvi, Rb = Ruskebukta, Sb = Skrukkebukta, Sg
889 = Stuora Galbajavri, Sj = Suolovjavri, St = Stuorajavri, Su = Suohpatjavri, Tb = Tjaerebukta, Va
890 = Vastusjärvi, Vd = Vuoddasjavri, Vg = Vuolgamasjavri, Vi = Virdnejavri, Vn = Vuolit Njivlojavri,
891 Vs = Vuolit Spielgajavri, Vu = Vuontisjärvi.

892

893 **Figure 2a.** Genotypic PCA of the 77 populations based on the 19 microsatellite loci. The first
894 axis representing the largest difference on the genetic data separated lakes from Alta
895 watercourse from the older watercourses, and was associated with the number of alleles in
896 populations, whereas the second axis was dividing lakes in Tana and Pasvik watercourses.
897 Inertia ellipses highlights where most of the individuals in each group are projected within the
898 axes. First explained 3% of the total variation, whereas the second axis explained 0.9% of the
899 total variation. Population abbreviations are combination of lake name (two first letters) and
900 eco-morph code (D = DR, LD = LDR, L = LSR, S = SSR).

901 **Figure 2b.** Genotypic PCA plot of each morphs across the three watercourses. Inertia ellipses
902 highlights where most of the individuals in each watercourses are projected within the axes.
903 DR, LDR, LSR and SSR morphs are coded with red, blue, yellow and black color, respectively.

904 **Figure 3.** Genotypic PCA of the 14 monomorphic populations based on the 19 microsatellite
905 loci. The first axis representing the largest difference on the genetic data was associated with
906 the number of alleles in populations, and explained 4.7% of the total variation, whereas the
907 second axis explained 2.3% of the total variation. Here the first axis separates populations in
908 the Alta watercourses from the two other watercourses, whereas second axis separates Tana
909 population from the Pasvik population. Population abbreviations are combination of lake name
910 (two first letters) and eco-morph code (L = LSR).

911 **Figure 4.** Summary plot of the phenotypic traits (i.e. weight and length of fish, number of gill
912 rakers, condition factor, and standardised length at age group) between each eco-morphs in
913 three watercourses. Populations in Alta, Tana and Pasvik watercourses are color coded as
914 red, blue and yellow. Violin plots displays the range of results, whereas the shape represent
915 the (mirrored) density plot, revealing where most of the results are located.

916 **Figure 5.** Phenotypic PCA calculated from the number of gill rakers, deviation from the mean
917 length per age group, and condition factor (All populations, monomorphic lakes included). The
918 different eco-morphs are color coded according to their watercourse (a). Figure b represent
919 eco-morphs in each of the three watercourses. First axis explained 44.1% of the total variation
920 and was associated with the size of fish. The second axis explained 30.9% of variation was
921 related to the number of gill rakers.

922 **Figure 6.** Landscape wide phenotypic divergence, estimated as Mahalanobis distance, plotted
923 against genetic divergence, estimated as pairwise G_{ST} and D distances. Markers where G_{ST}
924 underestimates divergence should have significantly elevated values of D. All the comparisons
925 were made eco-morph-wise against the ecomorph in question in lake Skrukkebukta. Only
926 exception was LDR populations, that were compared against DR, as LDR is not present in

927 Skrukkebukta. Dashed line is the overall regression across all populations (G_{ST} : $R^2 = 0.083$, P
928 $= 0.025$, D : $R^2 = 0.11$, $P = 0.009$). Here, we are not claiming causality but simply illustrating
929 the variation in current population.

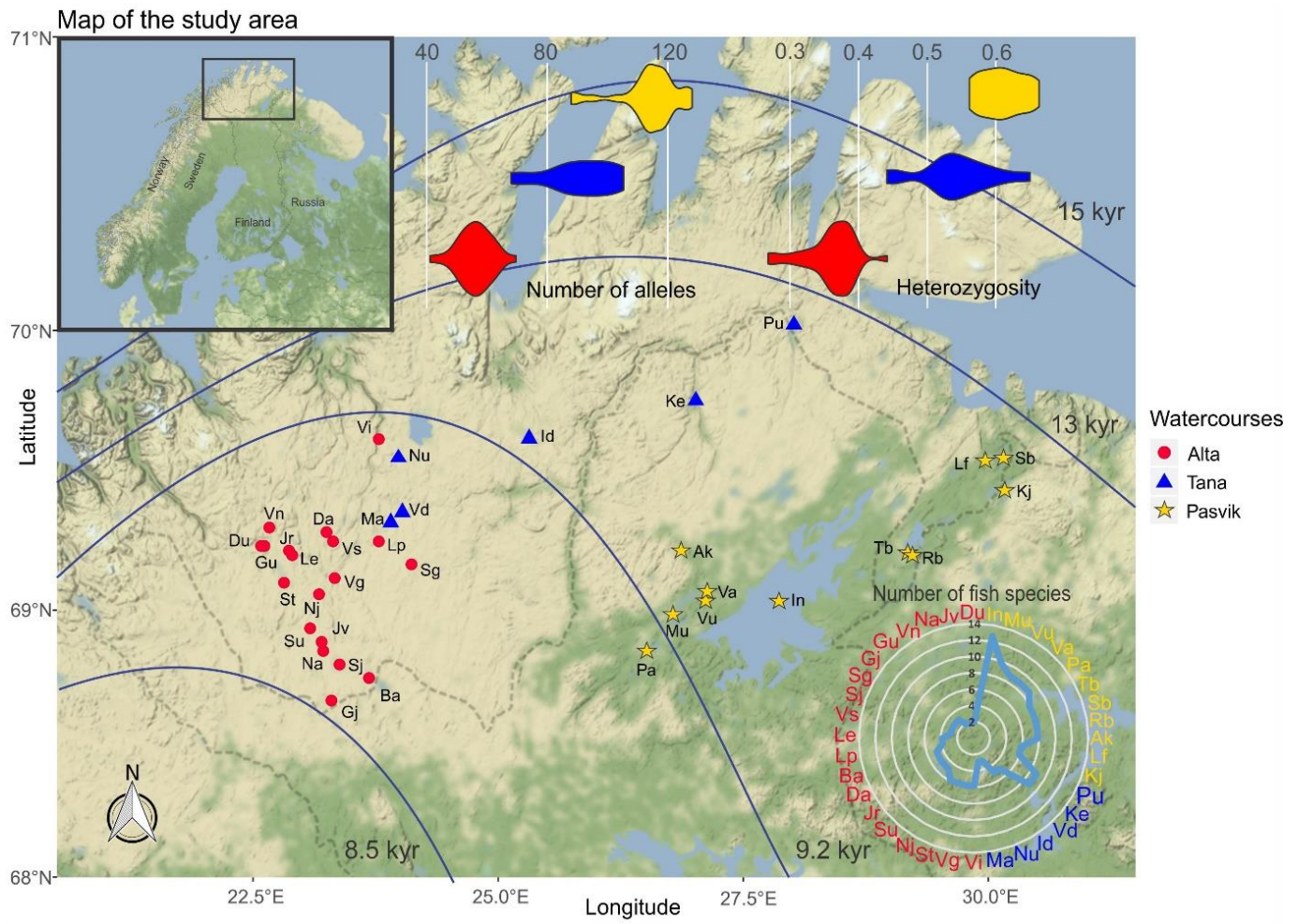
930 **Figure 7.** Pairwise comparison of G_{ST} and phenotypic distance between eco-morphs in each
931 lake among watercourses. Different shapes represent the three watercourses, and morph wise
932 comparisons in each lake are color-coded accordingly. Straight lines represents the regression
933 of each eco-morph pair comparison (DR-LSR: $R^2 = 0.21$, $P = 0.032$, $y=68.16x+8.73$, DR-SSR:
934 $R^2 = 0.53$, $P = 0.001$, $y=147.17x+8.97$, LSR-SSR: $R^2 = 0.65$, $P < 0.001$, $y=94.83x+1.57$, and
935 the overall regression across all comparisons $R^2 = 0.34$, $P < 0.001$, $y=159.01x+5.46$). As LDR
936 is present only in three lakes, regression lines are not presented for LDR comparisons.

937 **Figure 8.** Plot of the Individual based redundancy analysis (RDA) illustrating how phenotypic
938 variation reflects the underlying genetic variation. The RDA explained 11.7% of total inertia
939 (RDA1 7.9%, RDA2 1.4% $p < 0.001$). The first 10 PCA axis calculated from the allele
940 frequencies represented the genotypic data, and were plotted at the two first RDA axes (cf.
941 Figure 2). Arrows represent the direction and magnitude of the phenotypic traits loadings in
942 the RDA space. Individuals are color coded according to their original watercourses only for
943 illustrative purposes, as groupings were not included in the model.

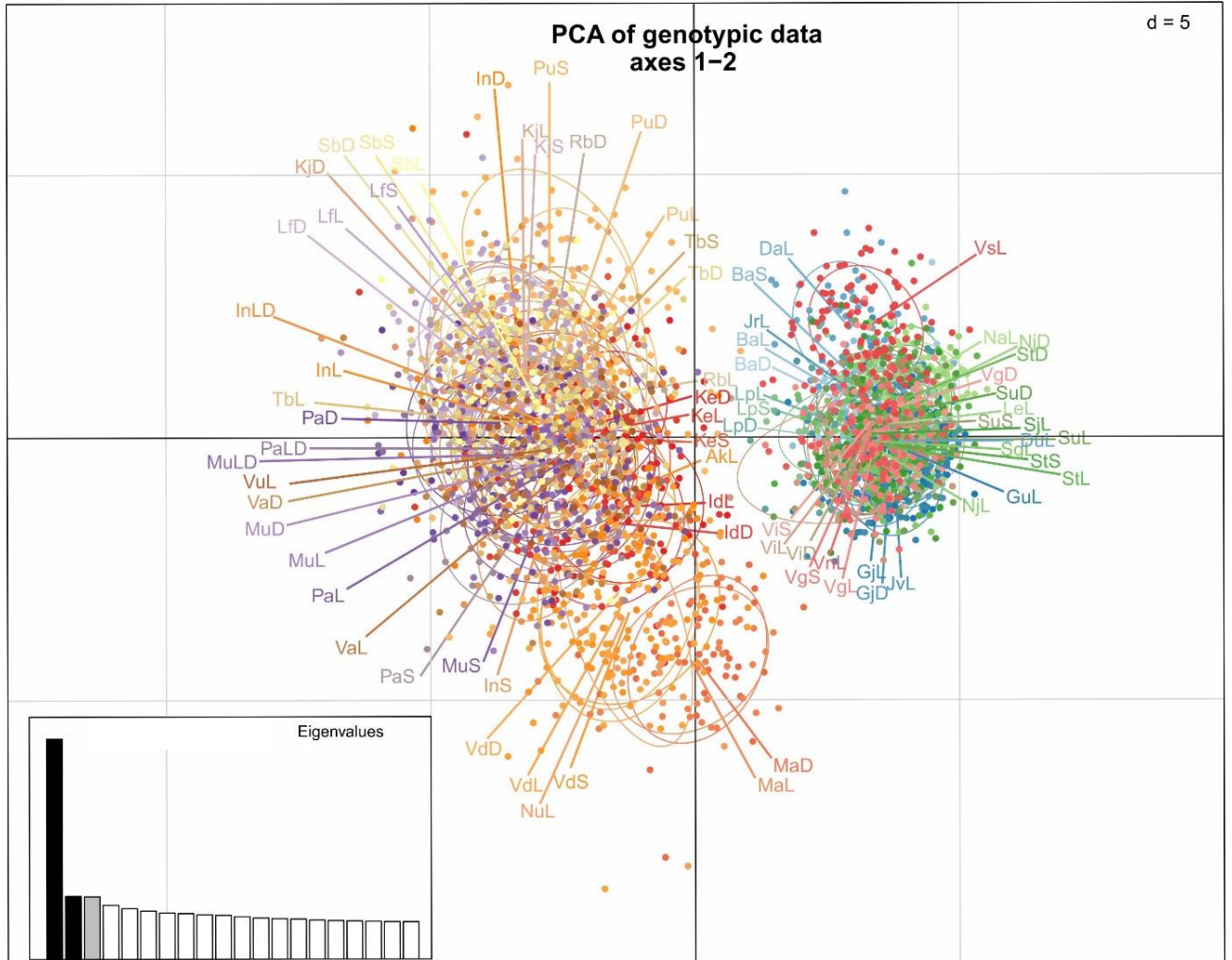
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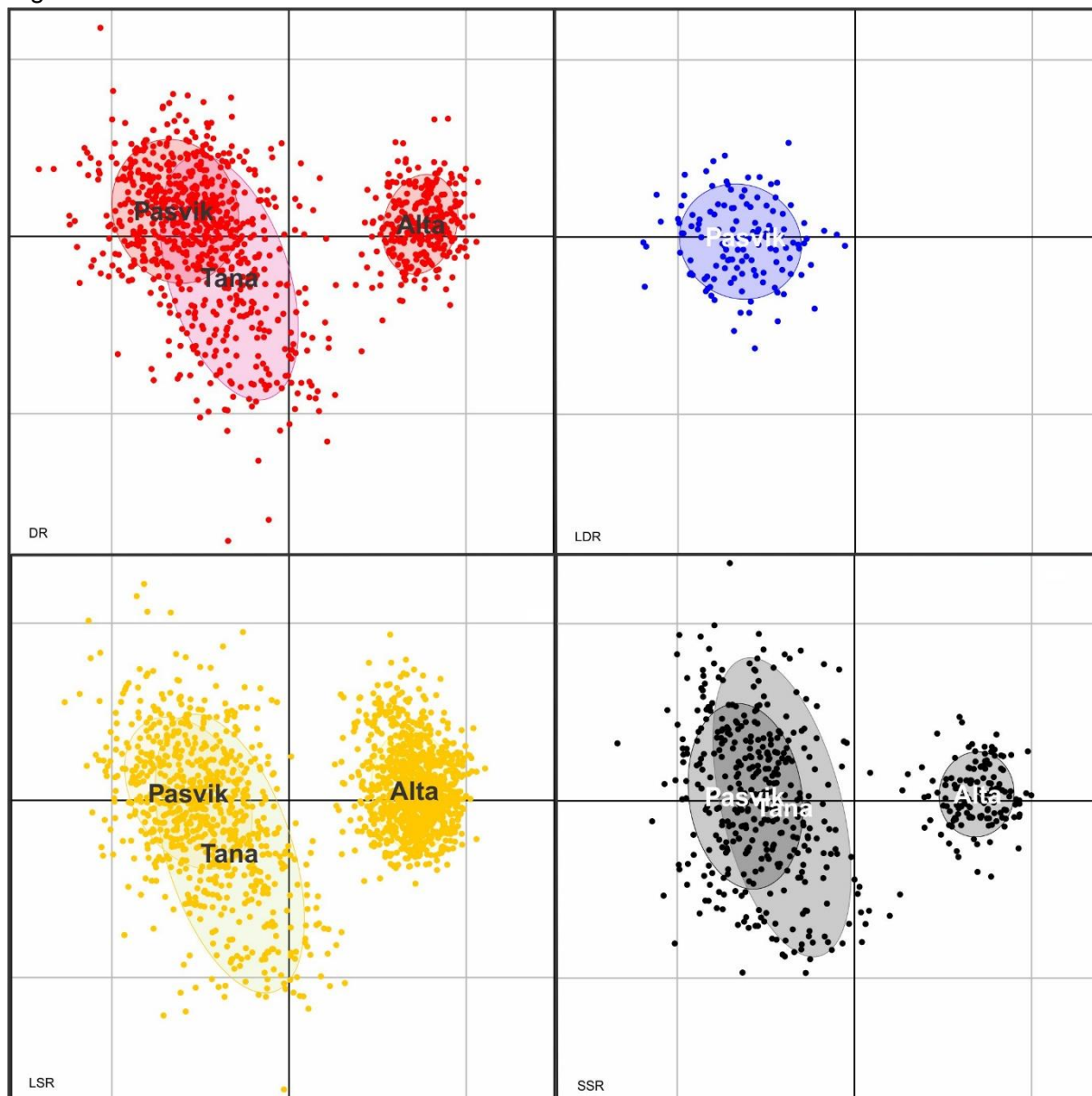
946 Figure 1.



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952 Figure 2b.

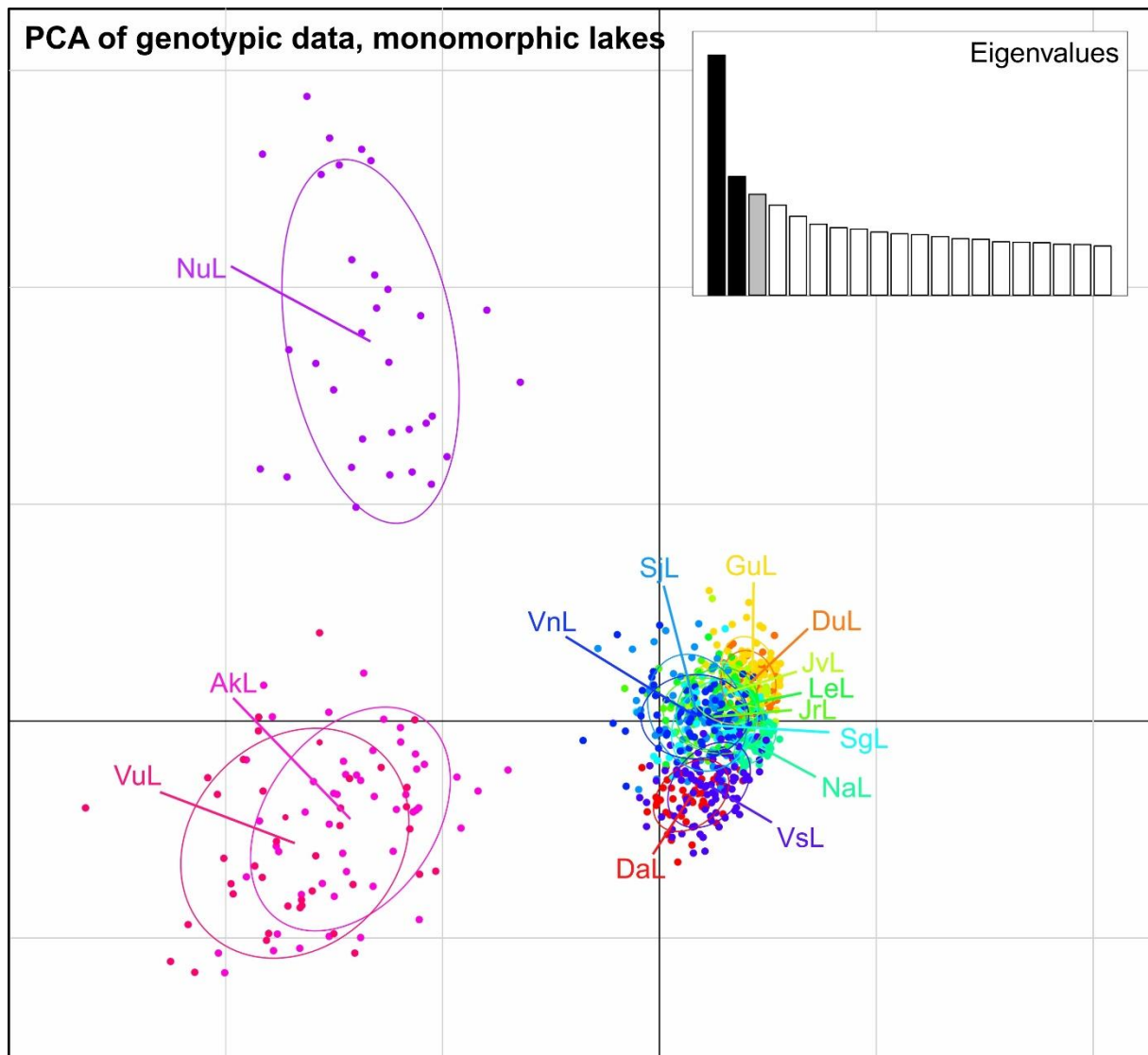


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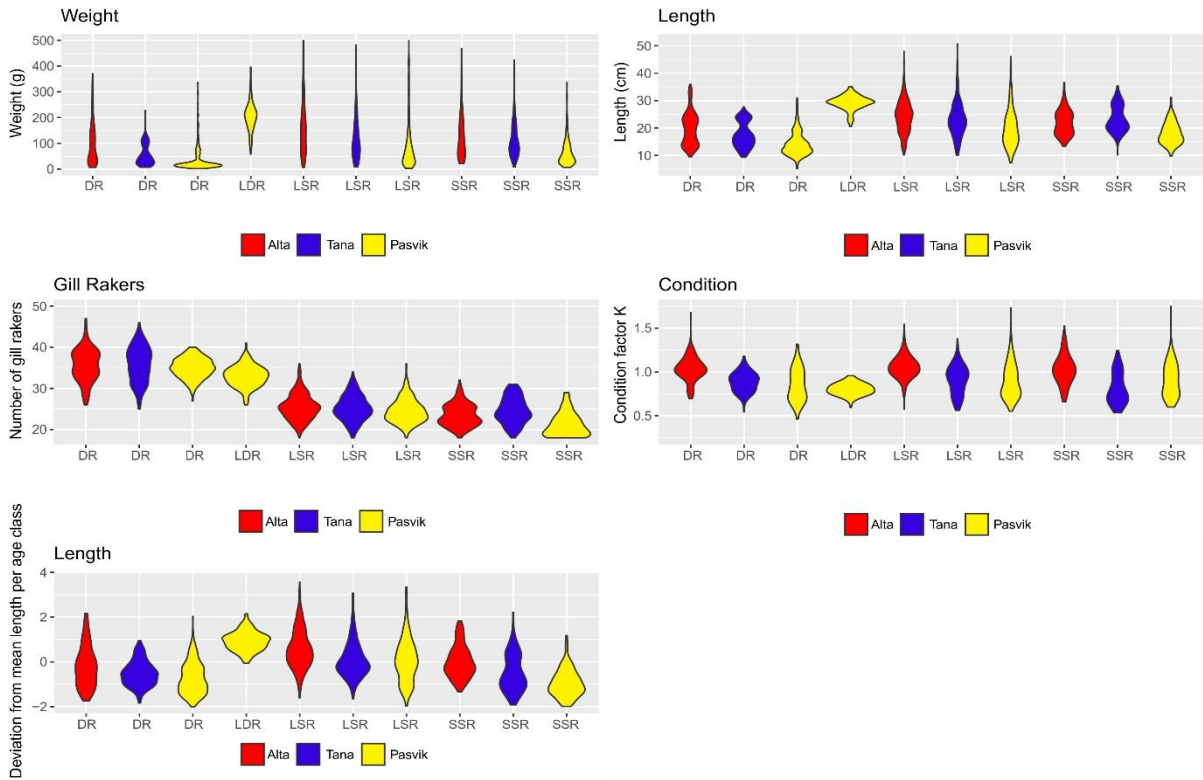
956 Figure 3.



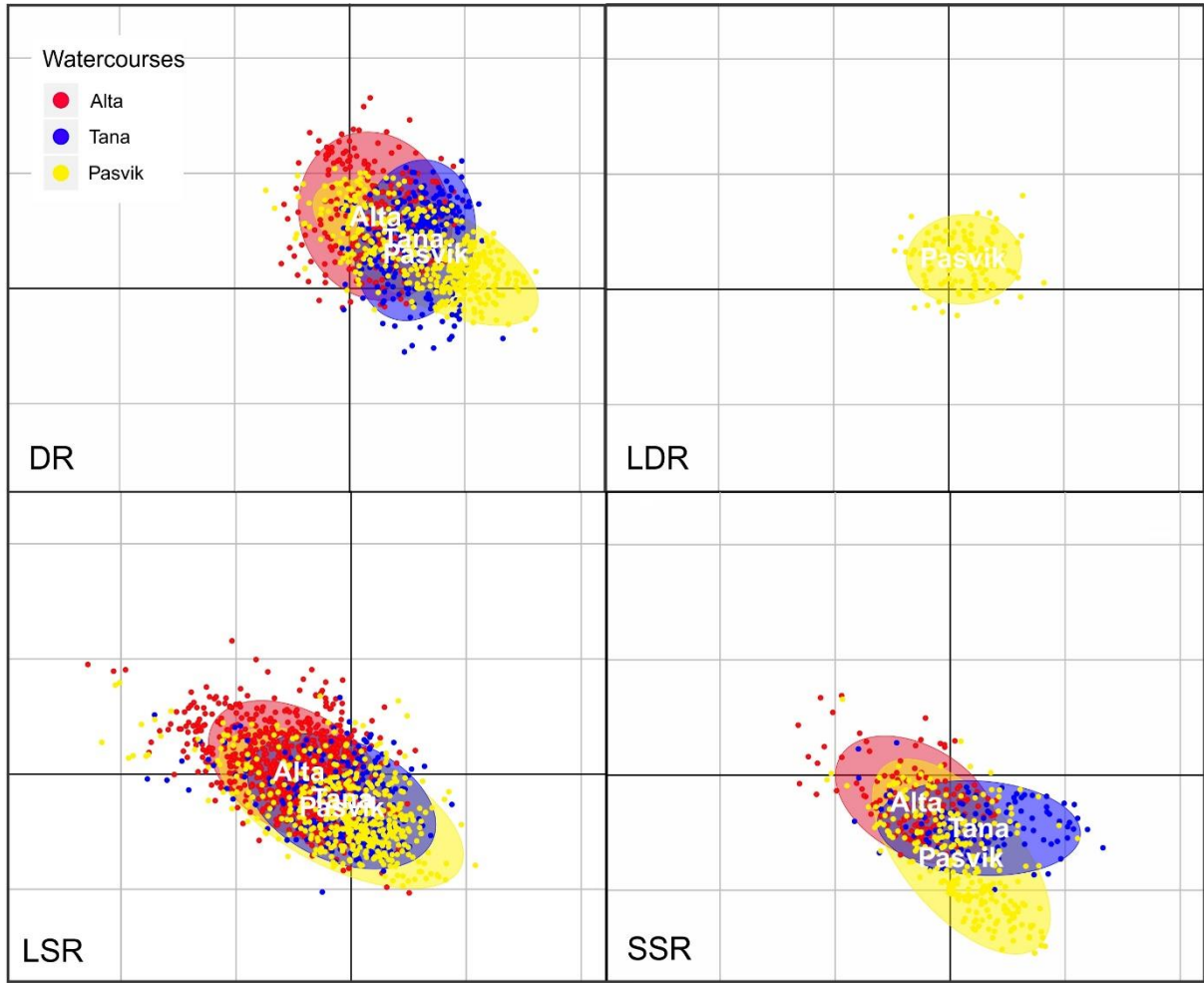
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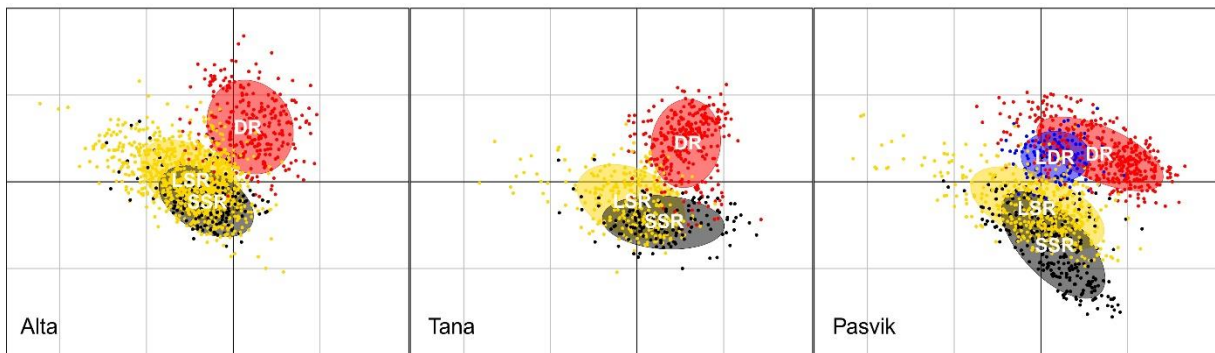
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962 Figure 5.



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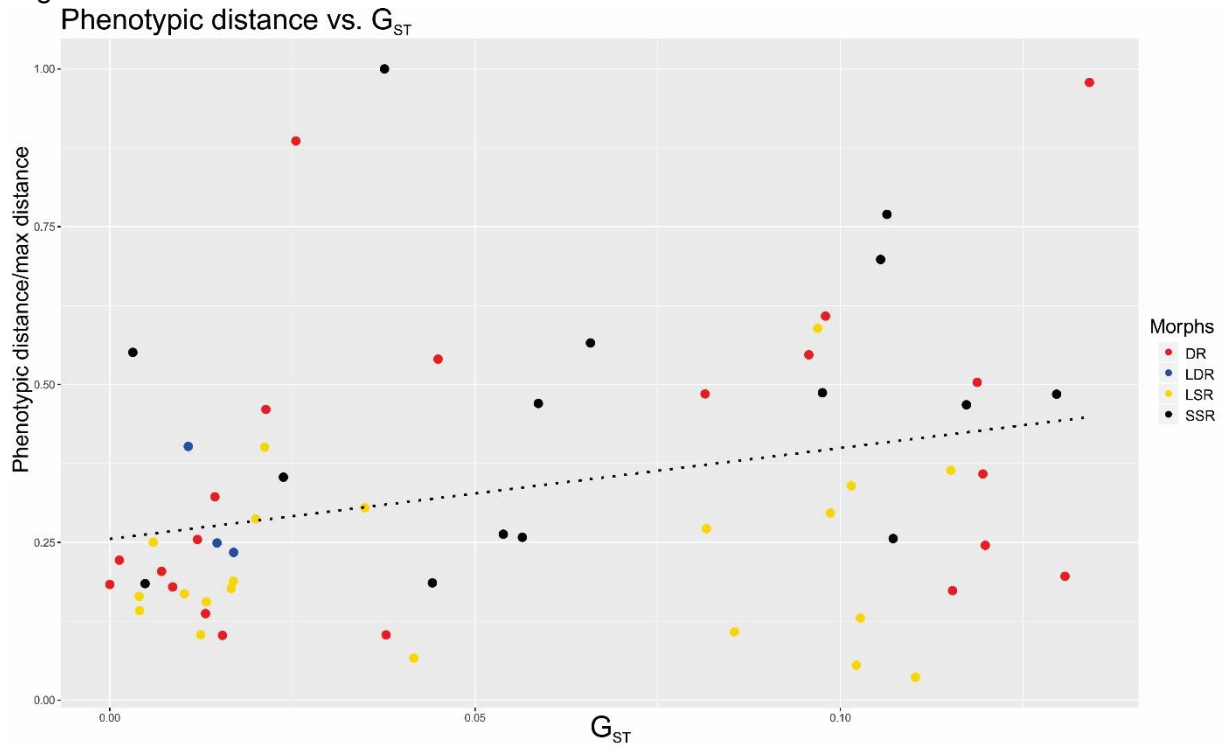
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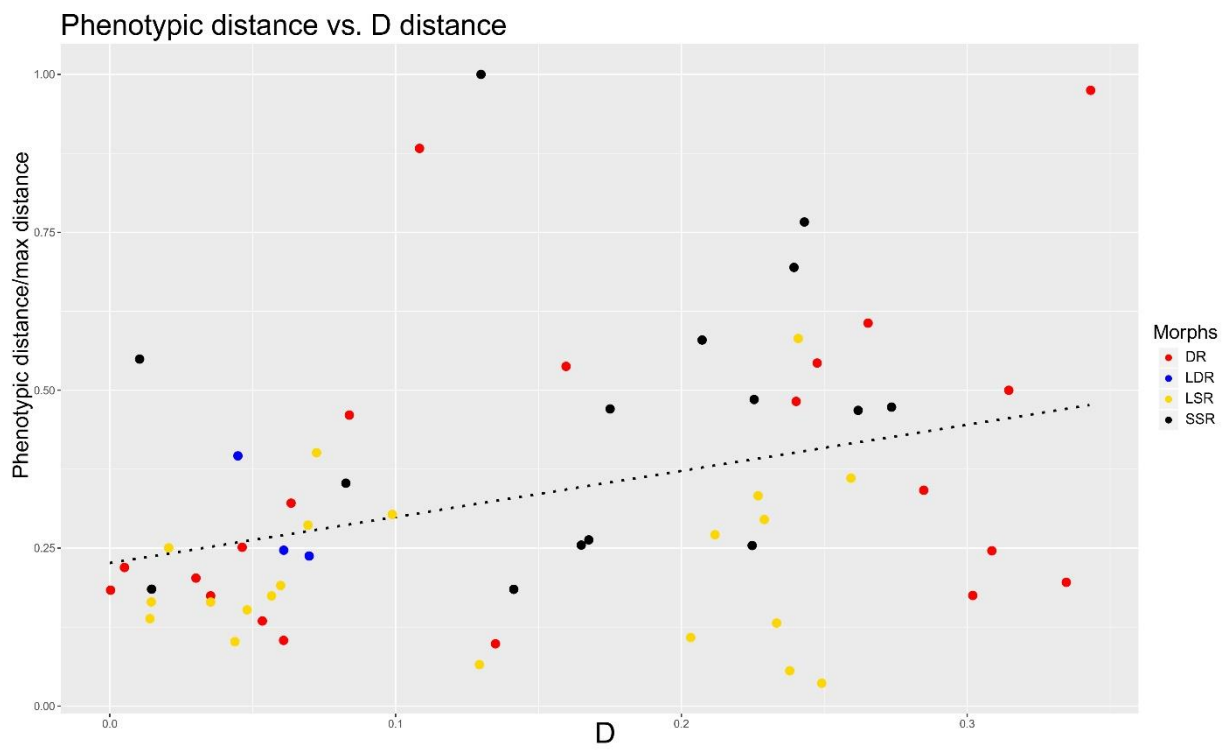
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968 Figure 6.



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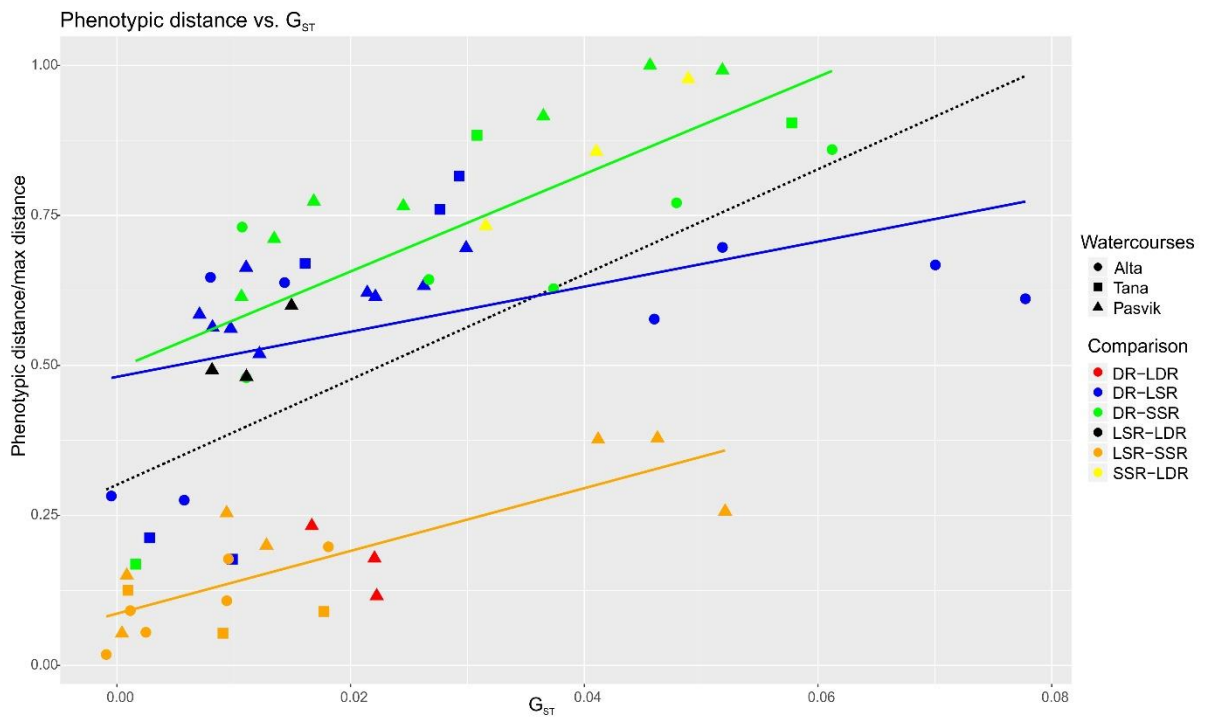


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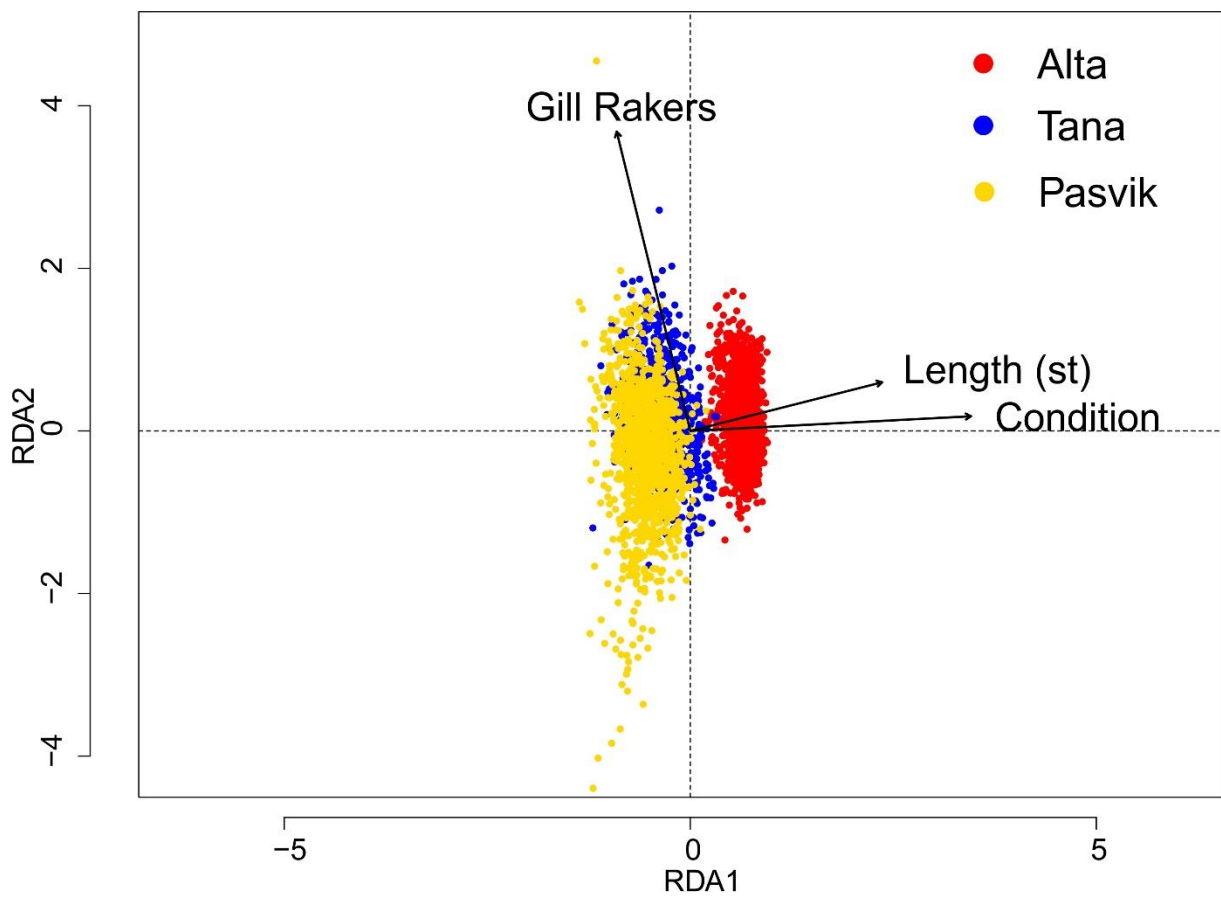
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973 Figure 7



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975 Figure 8.



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977 **Table 1.** Summary table of lake, lake code, watercourse, location, lake morphology, number
 978 of sampled eco-morphs, number of fish species, lake area and maximum depth.

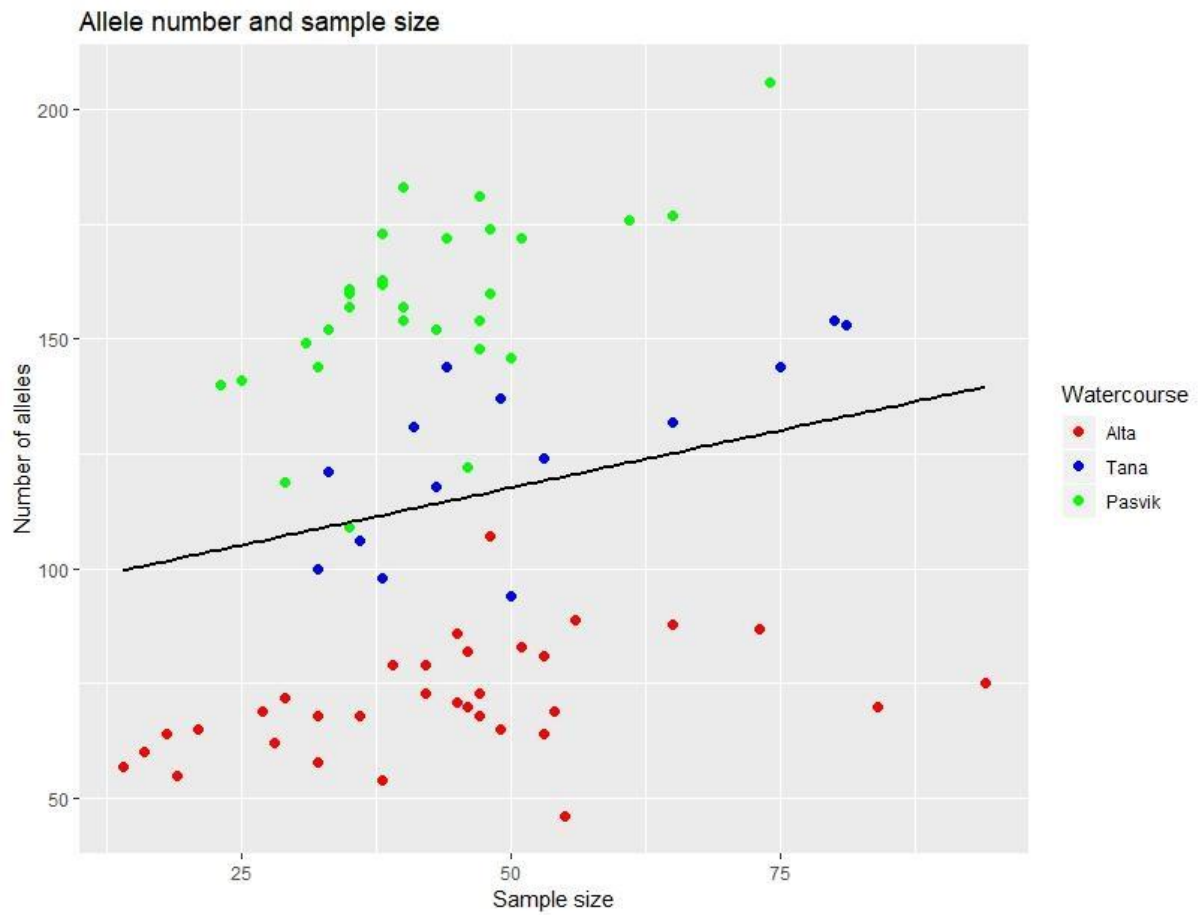
Lake	Code	Water course	Lat	Long	- morphic	Number of fish/morph				Number of fish species	area	max depth	Pairwise G_{ST}			
						DR	LDR	LSR	SSR				DR-LSR	DR-LDR	DR-SSR	LSR-SSR
Bajasjavri	Ba	Alta	68°45'	23°41'	tri	32	28	19	5	6.3	10	0.014		0.027	0.000	
Dátkojávri	Da	Alta	69°17'	23°15'	mono			49	5	4.2	17					
Durbunjavri	Du	Alta	69°14'	22°37'	mono			38	2	0.3	11					
Guorbajavri	Gu	Alta	69°14'	22°35'	mono			84	3	0.65	12					
Gædgejavri	Gj	Alta	68°40'	23°18'	di	36		54	3	4.25	20	0.006				
Jevdesjavri	Jv	Alta	68°53'	23°12'	mono			53	2	1.5	19					
Jårgajavri	Jr	Alta	69°13'	22°52'	mono			47	5	1.01	27					
Læmbejavri	Le	Alta	69°12'	22°54'	mono			47	4	0.4	6					
Lahpojavri	Lp	Alta	69°15'	23°47'	tri	45	45	18	4	8.12	36	0.008		0.011	0.001	
Nakkitjavri	Na	Alta	68°51'	23°13'	mono			55	2	0.78	17					
Njallajavri	Nj	Alta	69°03'	23°10'	di	46		51	6	2.1	30	0.078				
StuoraGalbajavri	Sg	Alta	69°10'	24°7'	mono			53	3	5.2	7					
Suolajavri	Sj	Alta	68°48'	23°23'	mono			65	3	7	7					
Stuorajavri	St	Alta	69°06'	22°49'	tri	46	39	27	6	23.7	30	0.046		0.037	0.002	
Suohpatjavri	Su	Alta	68°56'	23°05'	tri	32	42	21	5	2	25	0.052		0.061	0.010	
Vuolgamasjavri	Vg	Alta	69°07'	23°20'	tri	29	56	42	6	1.2	30	0.070		0.048	0.009	
Virdnejavri	Vi	Alta	69°37'	23°47'	tri	14	48	16	6	6.5	30	0.000		0.011	0.018	
Vuolit Njivlojavri Vuolit	Vn	Alta	69°18'	22°40'	mono			73	2	1.3	13					
Spielgajavri	Vs	Alta	69°15'	23°19'	mono			94	3	3.3	12					
Iddjajavri	Id	Tana	69°37'	25°19'	di	65		33	5	6.4	30	0.016				
Kevojärvi	Ke	Tana	69°45'	27°01'	tri	41	44	49	9	1.02	35	0.010		0.002	0.009	
Mohkkejavri	Ma	Tana	69°19'	23°54'	di	38		50	3	1.6	30	0.003				
Nuorbejavri	Nu	Tana	69°33'	23°59'	mono			32	3	2.3	NA					
Pulmankijärvi	Pu	Tana	70°01'	28°01'	tri	81	80	36	9	12	36	0.029		0.058	0.018	
Vuoddasjavri	Vd	Tana	69°21'	24°0'	tri	75	53	43	5	2.9	32	0.028		0.031	0.001	
Aksujärvi	Ak	Pasvik	69°13'	26°52'	mono			50	8	3.8	10					
Inarijärvi	In	Pasvik	69°02'	27°52'	tetra	35	38	35	29	13	1043	0.030	0.017	0.036	0.008	
Kuetsjarvi	Kj	Pasvik	69°26'	30°10'	tri	74		33	32	6	16	0.008		0.011	0.000	
Langfjordvatn	Lf	Pasvik	69°33'	29°58'	tri	31		65	47	6	2.8	0.010		0.013	0.001	
Muddusjärvi	Mu	Pasvik	68°59'	26°47'	tetra	38	47	48	46	10	48	0.021	0.022	0.052	0.046	
Paadarjärvi	Pa	Pasvik	68°51'	26°31'	tetra	40	43	35	35	9	21	0.026	0.022	0.046	0.041	
Ruskebukta	Rb	Pasvik	69°12'	29°13'	di	48	25		8	16.3	28	0.011		0.012	0.014	
Skrukkebukta	Sb	Pasvik	69°33'	30°06'	tri	47		61	40	8	6.6	0.012		0.025	0.009	
Tjaerebukta	Tb	Pasvik	69°13'	29°11'	tri	51		38	23	8	2.9	0.007		0.017	0.013	
Vastusjärvi	Va	Pasvik	69°04'	27°08'	di	48		44	9	4.3	15	0.022				
Vuontisjärvi	Vu	Pasvik	69°02'	27°07'	mono			40	9	11	31					

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981 **Supplementary figures**

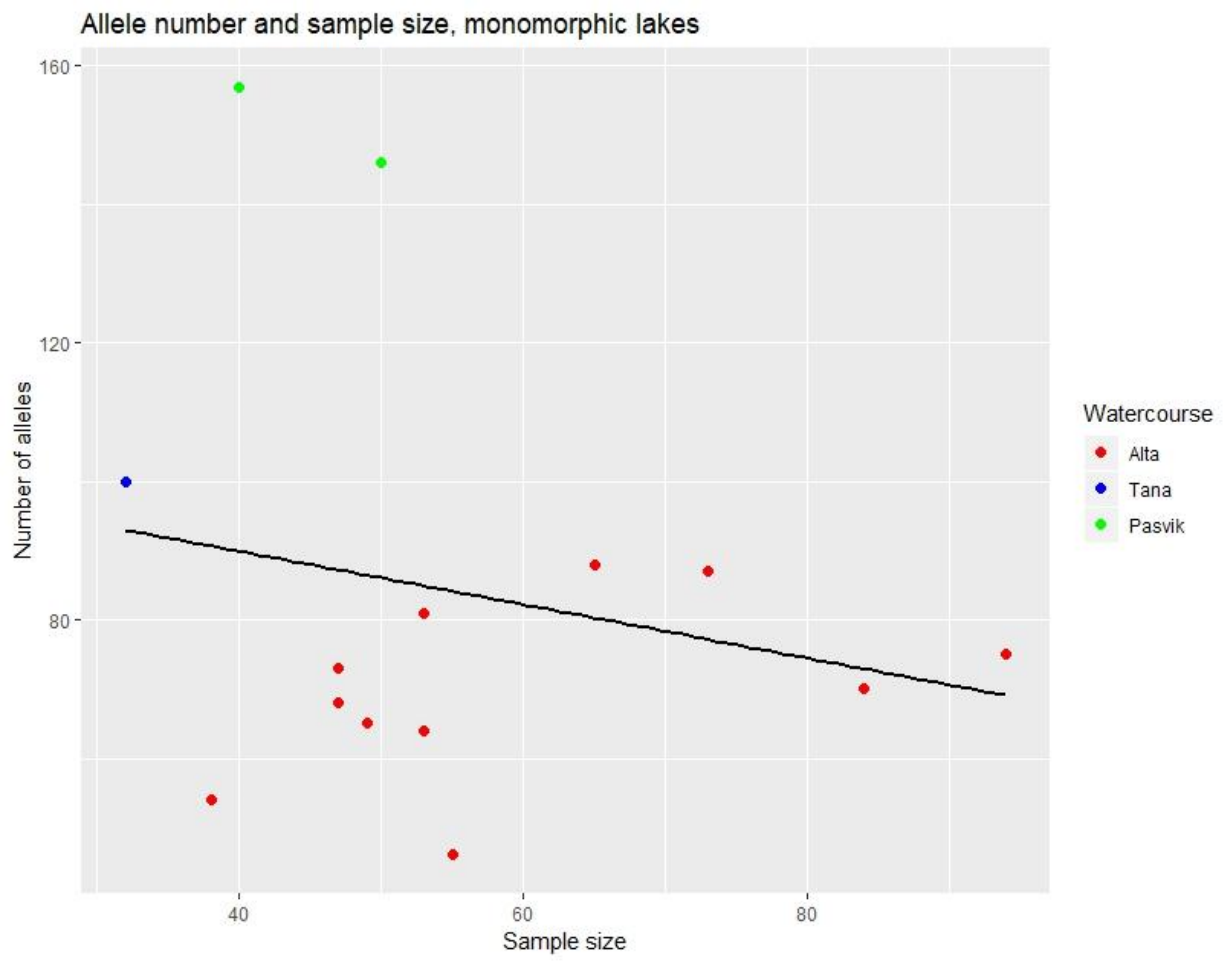
982 **Figure S1.** Sample size and number of alleles in each of the study populations. Populations in
983 Alta, Tana and Pasvik watercourses are color coded red, blue, and green, respectively.



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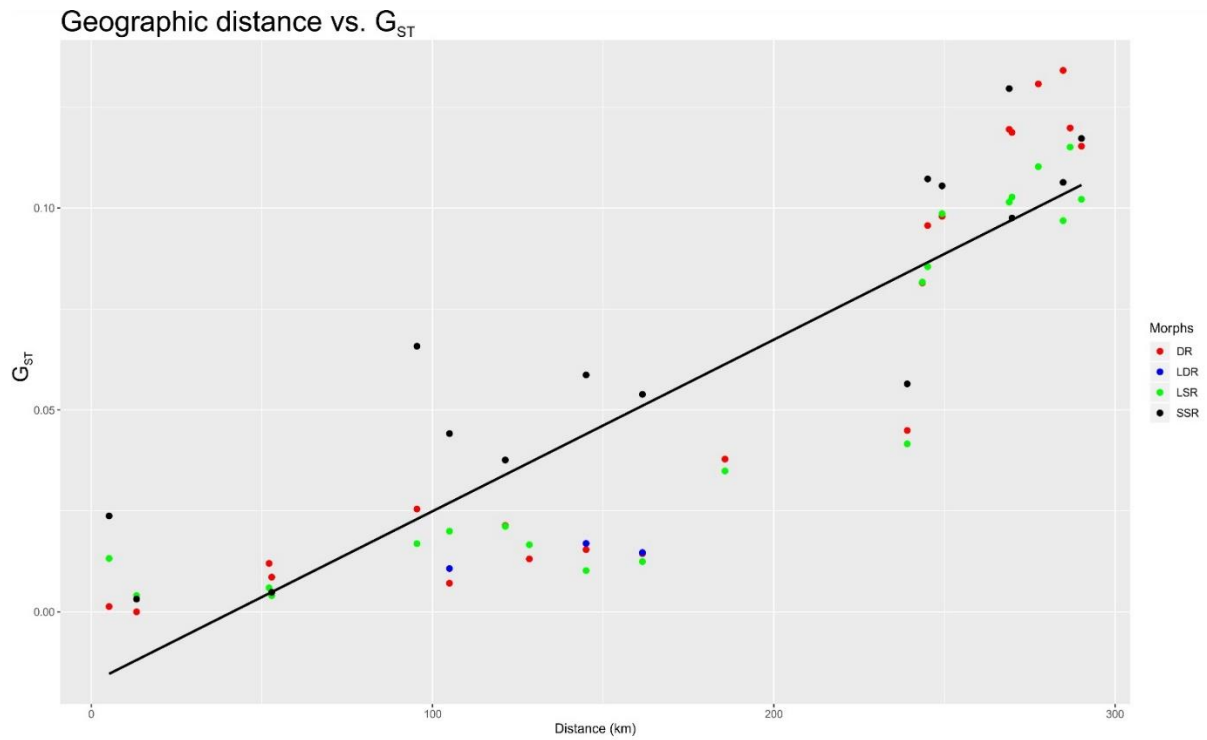
986 **Figure S2.** Number of alleles and sample size on monomorphic lakes. Populations in Alta,
987 Tana and Pasvik watercourses are color coded red, blue, and green, respectively.



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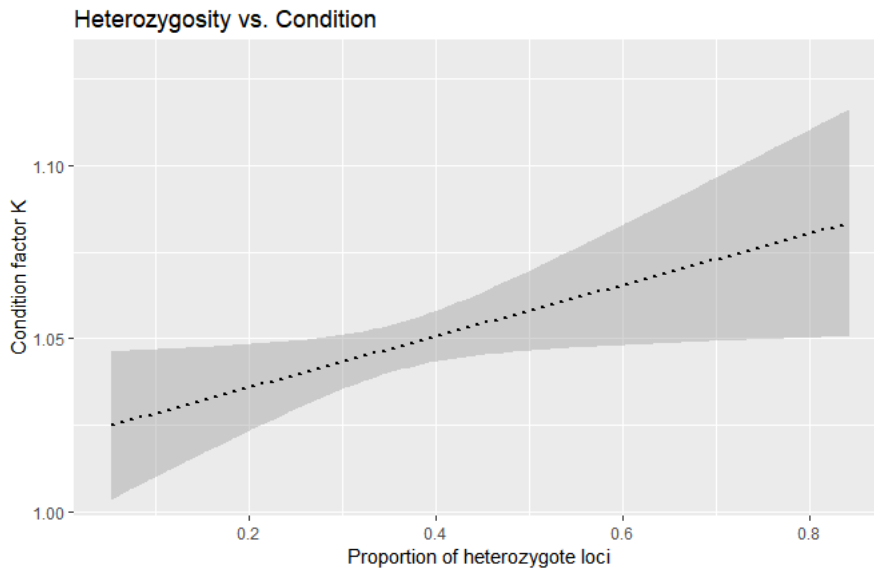
990 **Figure S3.** Association between geographic distance and G_{ST} . Pairwise G_{ST} plotted against
991 geographic distance from Skrukkebukta. All the comparisons were made morphwise against
992 the ecomorph in question in lake Skrukkebukta. Only exception was LDR populations that were
993 compared against DR, as LDR is not present in Skrukkebukta. Mantel test $r=0.72$, $p<0.001$



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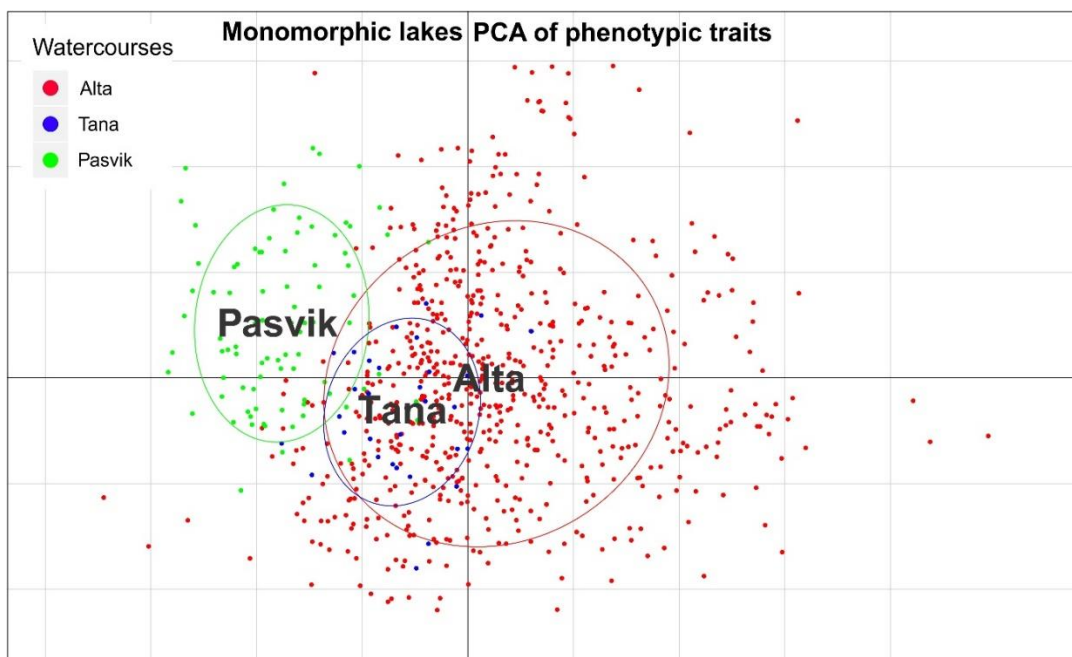
995

996 **Figure S4.** Association between K and heterozygosity in Alta watercourse. The linear
997 relationship ($p=0.03$) between proportion of heterozygote loci and condition factor K in Alta
998 watercourse. Dotted line represent the correlation and dark grey area around it is the
999 confidence interval.



1000

1001 **Figure S5.** Phenotypic PCA calculated from the number of gill rakers, deviation from the mean
1002 length per age group, and condition factor of LSR whitefish on monomorphic lakes. The
1003 different lake populations are color coded according to their watercourse. First axis explained
1004 50% and second 34% of variation.



1005

1006 **Table S1.** Summary table of total number of alleles in each loci, and per populations in the
 1007 three watercourses. For population abbreviations, see figure 2.

	Locus	Total	A l t a																			
			BaD	BaL	BaS	DaL	DuL	GjD	GjL	GuL	JvL	JrL	LpD	LpL	LpS	LeL	NaL	NjD	NjL	SgL	StD	StL
	BWF1	14	3	3	2	3	3	2	3	3	3	3	5	4	5	4	1	4	4	3	2	4
	BWF2	11	2	2	2	3	2	3	3	2	3	2	4	3	3	2	4	3	4	3	3	3
	Cla-Tet03	23	4	4	3	4	2	5	5	3	2	5	6	7	3	4	1	5	5	5	6	5
	Cla-Tet13	14	4	5	4	6	4	5	6	5	3	6	6	7	7	7	3	7	5	6	7	6
	Cla-Tet18	21	2	3	2	3	2	3	4	2	2	4	3	2	2	3	2	3	2	4	3	2
	Cocl-lav04	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
	Cocl-Lav06	15	2	2	2	2	2	2	1	4	3	2	4	2	2	2	2	3	3	2	2	
	Cocl-Lav10	5	3	3	2	3	2	3	3	2	1	3	3	3	2	3	2	3	3	2	3	3
	Cocl-lav27	10	2	2	2	2	2	1	1	3	2	2	1	1	1	2	1	2	2	1	3	3
	BFRO-018	10	1	1	1	1	1	1	1	2	2	2	2	1	1	2	1	1	1	1	1	1
	Cocl-lav18	8	2	2	2	2	1	2	2	1	2	2	2	2	2	1	2	2	2	2	3	2
	Cocl-lav49	18	2	2	2	2	3	3	2	3	2	4	2	2	3	4	2	3	4	4	4	3
	Cocl-lav52	39	3	5	3	9	5	8	6	6	6	5	10	4	4	6	5	6	7	8	9	7
	Cla-Tet06	39	6	5	6	5	7	5	5	6	6	6	8	5	4	5	4	5	7	10	6	6
	Cla-Tet09	18	7	7	6	5	5	6	8	8	6	7	8	8	8	5	6	6	10	10	7	8
	Cla-Tet15	13	4	5	4	3	3	2	2	3	4	5	4	4	3	4	3	3	5	4	5	5
	C2-157	26	1	2	1	1	2	2	2	2	2	1	3	2	1	1	1	1	2	1	3	4
	Cla-Tet01	21	3	2	2	6	4	4	5	6	5	6	6	4	4	6	2	4	8	3	6	5
	Cla-Tet17	35	6	6	8	4	3	10	9	8	9	7	8	9	8	6	3	9	8	9	8	9
	Sum	348	58	62	55	65	54	68	69	70	64	73	86	71	64	68	46	70	83	81	82	79
	Locus	StS	A l t a																			
			SjL	SuD	SuL	SuS	ViD	ViL	ViS	VgD	VgL	VgS	VnL	VsL								
	BWF1	4	4	3	4	3	2	6	3	3	5	4	3	2								
	BWF2	3	2	2	3	4	3	4	2	3	2	4	3	2								
	Cla-Tet03	6	7	4	4	3	6	6	3	5	4	4	5	5								
	Cla-Tet13	7	6	6	6	6	5	6	6	7	6	6	6	7								
	Cla-Tet18	3	2	2	2	2	3	3	3	3	3	3	4	4								
	Cocl-lav04	1	1	1	1	1	2	1	1	2	1	2	1	1								
	Cocl-Lav06	2	3	2	2	2	2	4	3	2	2	2	3	2								
	Cocl-Lav10	3	3	3	3	3	3	3	3	3	3	4	3	3								
	Cocl-lav27	2	3	2	2	2	1	1	1	1	3	2	3	2								
	BFRO-018	1	2	1	2	1	1	2	1	1	1	1	1	1								
	Cocl-lav18	1	2	2	2	2	2	2	2	3	2	3	2	2								
	Cocl-lav49	3	4	4	3	3	3	6	3	3	4	3	5	5								
	Cocl-lav52	9	11	7	6	5	3	11	3	7	8	6	10	8								
	Cla-Tet06	4	8	5	7	4	5	12	4	5	8	8	6	4								
	Cla-Tet09	4	10	7	8	7	5	10	6	8	10	7	6	8								
	Cla-Tet15	4	4	4	4	4	3	5	2	3	5	5	6	5								
	C2-157	1	2	1	2	1	1	3	1	2	3	4	1	1								
	Cla-Tet01	5	6	4	6	5	5	8	5	5	8	5	7	6								
	Cla-Tet17	6	8	8	6	7	4	13	8	8	9	9	10	7								
	Sum	69	88	68	73	65	57	107	60	72	89	79	87	75								
	Locus	IdD	T a n a																			
			IdL	KeD	KeL	KeS	MaD	MaL	NuL	PuD	PuL	PuS	VdD	VdL	VdS							
	BWF1	5	5	6	7	8	5	5	4	9	7	6	6	6								
	BWF2	4	3	5	6	5	3	3	5	5	4	4	4	5								
	Cla-Tet03	9	8	9	10	9	6	7	6	16	11	8	9	8								
	Cla-Tet13	8	9	11	11	8	4	8	7	7	9	7	8	7								
	Cla-Tet18	5	3	5	7	7	2	3	3	4	5	4	5	3								
	Cocl-lav04	5	2	3	4	4	1	2	5	4	5	3	3	2								
	Cocl-Lav06	6	6	6	7	5	5	3	3	8	6	5	7	6								
	Cocl-Lav10	2	2	3	3	3	3	3	3	4	3	3	3	3								
	Cocl-lav27	3	2	4	4	3	1	1	2	5	5	2	4	4								
	BFRO-018	4	5	4	6	5	1	3	3	4	5	4	1	2								
	Cocl-lav18	3	4	4	3	3	2	2	2	4	2	2	2	2								
	Cocl-lav49	4	5	7	8	9	5	5	3	6	5	6	12	6								
	Cocl-lav52	9	10	7	12	7	11	7	8	12	11	7	13	13								
	Cla-Tet06	11	9	11	13	13	16	13	17	17	15	12	15	14								
	Cla-Tet09	10	10	8	11	11	7	4	8	13	14	8	11	7								
	Cla-Tet15	4	4	4	6	4	4	3	4	5	4	3	4	3								
	C2-157	12	8	7	7	9	5	6	5	7	11	7	7	6								
	Cla-Tet01	9	6	9	8	9	5	5	4	11	9	6	11	7								
	Cla-Tet17	19	20	18	11	15	12	11	8	15	19	9	19	16								
	Sum	132	121	131	144	137	98	94	100	153	154	106	144	124								
	Locus	AkL	T a n a																			
			InD	InL	InLD	InS	KjD	KjL	KjS	LfD	LfL	LfS	MuD	MuL	MuLD	MuS	PaD	PaL	PaLD	PaS	RbD	RbL
	BWF1	8	6	8	7	6	7	7	6	7	7	7	7	8	7	7	8	8	7	6	8	7
	BWF2	6	6	7	6	4	9	9	5	6	8	6	6	5	5	6	7	5	5	4	7	6
	Cla-Tet03	9	10	11	10	10	11	10	8	7	13	9	10	10	7	12	13	9	8	10	10	8

P a s s i v i k	Cla-Tet13	8	9	8	10	6	11	10	8	8	10	10	9	10	7	6	9	7	8	5	10	7	
	Cla-Tet18	6	6	12	12	5	10	8	7	5	7	7	6	10	6	5	8	11	11	3	7	6	
	Cocl-lav04	2	4	5	6	3	6	4	3	2	3	3	4	6	4	4	6	5	5	3	4	4	
	Cocl-Lav06	4	8	7	8	6	8	5	6	7	7	8	6	6	6	6	8	6	8	6	6	8	
	Cocl-Lav10	3	4	4	3	3	3	3	3	3	3	3	3	4	3	3	3	3	3	2	3	3	
	Cocl-lav27	3	3	1	4	1	5	3	3	3	5	3	5	3	2	1	4	3	2	1	4	4	
	BFRO-018	3	4	3	5	2	5	5	4	4	6	6	3	4	4	2	3	5	5	2	5	5	
	Cocl-lav18	3	2	2	2	2	2	2	2	2	2	2	2	3	2	3	2	3	2	2	2	3	2
	Cocl-lav49	6	6	7	8	8	10	6	9	7	7	7	7	6	6	5	6	8	7	6	5	7	8
	Cocl-lav52	15	16	12	16	14	21	11	14	15	18	17	18	16	14	13	18	14	16	11	11	11	11
	Cla-Tet06	19	17	15	17	12	24	14	13	16	19	15	21	22	17	12	20	22	14	11	17	10	10
	Cla-Tet09	10	14	9	12	8	12	12	12	11	13	10	12	13	13	9	12	10	11	9	12	12	12
	Cla-Tet15	4	6	6	8	3	6	4	4	4	6	4	6	5	3	4	6	4	3	3	4	5	5
	C2-157	9	9	10	9	7	12	10	11	8	10	9	9	10	10	6	10	9	8	6	12	8	8
	Cla-Tet01	15	13	13	13	9	17	12	11	15	13	12	12	13	12	12	16	11	12	9	13	11	11
	Cla-Tet17	13	14	20	17	10	27	17	15	19	20	16	17	21	20	6	21	20	18	11	17	16	16
	Sum	146	157	160	173	119	206	152	144	149	177	154	163	174	148	122	183	161	152	109	160	141	141
	P a s s i v i k	Locus	SbD	SbL	SbS	TbD	TbL	TbS	VaD	VaL	VuL												
		BWF1	7	7	7	7	6	7	7	8	7												
		BWF2	8	7	7	7	7	8	7	6	7												
Cla-Tet03		12	12	10	10	9	10	9	7	10													
Cla-Tet13		10	8	7	10	7	4	10	8	6													
Cla-Tet18		8	6	6	6	6	4	7	8	6													
Cocl-lav04		4	5	5	4	4	3	5	5	4													
Cocl-Lav06		7	8	7	7	8	6	7	6	7													
Cocl-Lav10		3	3	3	3	3	3	3	3	3													
Cocl-lav27		5	5	2	4	5	4	4	3	5													
BFRO-018		5	5	4	5	5	5	2	3	5													
Cocl-lav18		2	2	3	2	2	2	3	2	2													
Cocl-lav49		6	9	8	7	7	7	6	6	8													
Cocl-lav52		18	12	13	15	18	10	18	15	19													
Cla-Tet06		19	18	14	22	16	15	19	20	8													
Cla-Tet09		13	13	12	13	11	11	12	13	15													
Cla-Tet15		5	5	5	4	4	4	4	6	7	5												
C2-157	11	12	10	11	9	9	11	11	10														
Cla-Tet01	15	16	13	16	14	10	15	16	15														
Cla-Tet17	23	23	18	19	21	18	23	25	15														
Sum	181	176	154	172	162	140	174	172	157														

1009 **Table S2.** Summary trait table of the populations indicating mean number of gill rakers, mean
 1010 total length (cm) of fish, and mean weight \pm standard deviation (SD) and minimum and
 1011 maximum. For population abbreviations, see figure 2.

Lake	Pop	Mean Gill Rakers \pm SD (min-max)	Mean Length \pm SD (min-max)	Mean Weight \pm SD (min-max)
Aksujärvi	AkL	24.3 \pm 1.4 (21-27)	23.4 \pm 3.8 (14.1-33)	108.2 \pm 53 (19.9-319.4)
Bajasjavri	BaD	33.9 \pm 2.6 (28-39)	28.4 \pm 6.2 (15.7-35.9)	207.7 \pm 112.7 (31-370)
	BaL	22.5 \pm 1.2 (20-25)	29.1 \pm 7.1 (15.1-39.2)	246.3 \pm 159.8 (25-561)
	BaS	22.4 \pm 1.2 (20-26)	18.4 \pm 4.6 (14.4-30.6)	57.6 \pm 56.7 (21-220)
Dátkojávri	DaL	28.1 \pm 1.9 (24-33)	25.4 \pm 3.1 (19.3-32.3)	170.9 \pm 59.7 (63.9-338)
Durbunjavri	DuL	25.3 \pm 1.6 (21-29)	25.8 \pm 6.5 (19.5-48)	258.7 \pm 360.1 (69-1670)
Gædgejavri	GjD	32.1 \pm 2.8 (26-37)	14.8 \pm 1.9 (12.8-22.5)	34.3 \pm 20.2 (21.4-130)
	GjL	27.1 \pm 2.2 (23-34)	21.7 \pm 3 (13.8-29.5)	121.6 \pm 55.3 (23.8-319.3)
Guorbajavri	GuL	24.5 \pm 1.5 (21-29)	22.6 \pm 7.5 (11.5-42.8)	163.1 \pm 152.6 (14.1-1000)
Iddjavri	IdD	34.3 \pm 2 (30-39)	17.4 \pm 3.1 (10.4-27.7)	58 \pm 37.8 (9.6-228)
	IdL	22.2 \pm 3.2 (15-34)	22.6 \pm 7.5 (10.2-40.7)	167.5 \pm 179.8 (10.1-930)
	Inari	InD	35.4 \pm 2.5 (29-39)	12 \pm 3.1 (6.1-19.5)
Inari	InL	22.8 \pm 2.8 (19-32)	26.2 \pm 7.1 (14.4-46.1)	194.1 \pm 187.7 (22.4-1005.5)
	InLD	31.6 \pm 2.6 (26-41)	28.8 \pm 3.5 (20.5-35)	212.5 \pm 76.6 (58.4-395.6)
	InS	18.8 \pm 2.6 (16-28)	21 \pm 3.2 (13.9-25.3)	73.3 \pm 30.3 (19.8-130.1)
	Jårgajavri	JrL	24.6 \pm 2.4 (19-31)	24.5 \pm 5.3 (10.2-34.4)
Jevdesjavri	JvL	25.5 \pm 3.1 (19-30)	18.9 \pm 3.1 (12.1-27.9)	75.6 \pm 39.8 (21-239)
Kevo	KeD	30.5 \pm 2.4 (25-35)	15.4 \pm 2.8 (10.1-21.3)	31.1 \pm 16.3 (8-76.6)
	KeL	27.3 \pm 2.2 (23-32)	23.4 \pm 5.6 (10.1-38.8)	138 \pm 109.9 (8-652.6)
	KeS	27.4 \pm 2.5 (19-31)	21.6 \pm 3.4 (15.8-35.4)	80.8 \pm 51.7 (21.1-354.5)
	Kuetsjarvi	KjD	35.2 \pm 2.6 (27-40)	11.9 \pm 3.8 (8.8-28.2)
Kuetsjarvi	KjL	25 \pm 3.3 (19-32)	13.7 \pm 2.8 (9.6-20.5)	28.7 \pm 20.9 (7-99)
	KjS	24.2 \pm 3.2 (19-29)	13.8 \pm 2.1 (9.8-18.4)	26.5 \pm 14 (7-64)
	Læmbejavri	LeL	23.2 \pm 1.8 (20-27)	30.6 \pm 3.7 (23.3-37)
Langfjordvatn	LfD	35.4 \pm 3 (27-40)	12.1 \pm 1 (11.2-16.3)	19.6 \pm 6.2 (12-45.1)
	LfL	25.3 \pm 3.4 (20-36)	17.8 \pm 4.5 (10.9-35.1)	80.4 \pm 97.2 (13-640.1)
	LfS	22.6 \pm 2.4 (17-29)	20.2 \pm 2.8 (15.5-29.5)	99.8 \pm 51.4 (37-336.3)
Lahpojavi	LpD	38.3 \pm 2.4 (31-43)	13.1 \pm 1.8 (11.5-20.5)	25.7 \pm 17.1 (15.2-100.8)
	LpL	26.6 \pm 2.6 (22-32)	21.8 \pm 3.3 (16-31.5)	130.2 \pm 67.9 (48.4-423)
	LpS	25.1 \pm 1.6 (22-29)	21.8 \pm 2.9 (13.3-25.6)	120.6 \pm 49.4 (24-228)
Mohkkejavri	MaD	31 \pm 1.8 (26-34)	15.6 \pm 2.2 (10.9-20.7)	38.4 \pm 16.3 (11.9-92.6)
	MaL	26.4 \pm 3.9 (18-33)	22 \pm 7 (10.9-38.2)	158 \pm 168.4 (12.3-687.4)
Muddusjärvi	MuD	34.5 \pm 2 (31-38)	13.6 \pm 0.8 (11.8-15.2)	17.6 \pm 3.8 (10.6-25.1)
	MuL	23.3 \pm 1.8 (20-29)	14.6 \pm 3.6 (9.4-21.6)	26.3 \pm 19.1 (4.6-75.4)
	MuLD	33.9 \pm 2.2 (30-38)	29.3 \pm 2.7 (22.4-34.3)	197.8 \pm 55.8 (88.6-343)
	MuS	16.8 \pm 1.2 (14-19)	15.3 \pm 1.2 (13-18.6)	25.7 \pm 6.9 (16.8-52)
Nakkitjavri	NaL	21 \pm 1.1 (19-23)	21.8 \pm 2.7 (17-28.9)	118.2 \pm 56.8 (47.4-317.8)
Njallajavri	NjD	35.2 \pm 3.1 (27-42)	15.7 \pm 2.2 (9.5-19.4)	41.6 \pm 15.5 (7-67)
	NjL	24.2 \pm 2.1 (18-31)	20.4 \pm 6.3 (11-37)	116.7 \pm 129.8 (12-667)
Nuorbejavri	NuL	24.7 \pm 1.9 (20-28)	23.8 \pm 3 (18.2-29.5)	145.5 \pm 56 (62-276)

Paadar	PaD	36.2 ± 1.8 (33-40)	13.9 ± 1.3 (11.3-16.4)	19.6 ± 5.6 (10-31)
	PaL	24.7 ± 2.7 (20-32)	18.5 ± 4.6 (12.2-27)	54.8 ± 41.4 (13-160.1)
	PaLD	33.3 ± 2.4 (27-38)	29.4 ± 1.8 (21.6-32.1)	205.2 ± 30.6 (85.3-252.6)
Pulmanki	PaS	18.2 ± 1.5 (15-21)	20.6 ± 4.9 (11-31.2)	82.6 ± 66.6 (10.3-279.4)
	PuD	40.1 ± 2.3 (36-46)	23.3 ± 2.3 (12.8-26.2)	103.7 ± 24.5 (12.5-136.7)
	PuL	25.4 ± 2.3 (22-31)	25.5 ± 6.8 (13.5-50.7)	156.6 ± 177.8 (15.8-1182.7)
Ruskebukta	PuS	23.9 ± 1.7 (20-28)	28.7 ± 2.5 (20.8-32.7)	163.7 ± 39.6 (66.2-258.5)
	RbD	35.4 ± 2.4 (31-40)	16.5 ± 4.6 (7.5-25.3)	58 ± 46.1 (3.8-173)
	RbL	23.4 ± 2.4 (18-29)	25.6 ± 12.9 (7.4-41.9)	365.7 ± 367 (3.8-939)
Skrukkebukta	SbD	33.8 ± 2.6 (29-40)	12.3 ± 3.4 (6.2-22.9)	22.1 ± 25.3 (2-126)
	SbL	24.5 ± 3 (18-33)	18.1 ± 5.4 (7.7-35.4)	78.1 ± 85.7 (4-543)
	SbS	20.1 ± 1.8 (16-23)	16.3 ± 1.9 (12-21.6)	43.6 ± 16.6 (15.6-104)
StuoraGalbajavri	SgL	22.5 ± 1.3 (19-25)	21.5 ± 5.4 (11.8-31.2)	113.8 ± 75.9 (13.8-320)
Suolajavri	SjL	26.3 ± 2.9 (20-35)	27.4 ± 5.3 (16.3-37.1)	266.8 ± 135.4 (43-613)
Stuorajavri	StD	34.5 ± 2.6 (26-40)	21.9 ± 2.9 (13.1-29.5)	111.6 ± 45.5 (19.1-299)
	StL	24.2 ± 1.9 (21-28)	21 ± 5.3 (14-33.7)	115.7 ± 108.4 (25.2-450)
	StS	23.3 ± 2.2 (19-26)	19.7 ± 3.8 (14-30.1)	85.8 ± 65.7 (23.7-321.5)
Suohpatjavri	SuD	40.8 ± 2.6 (37-47)	24 ± 1.1 (21.8-25.5)	160.4 ± 24.1 (109.8-197)
	SuL	28.2 ± 2 (24-32)	23.8 ± 6 (16.5-35.5)	186.1 ± 155.2 (50.8-603.6)
	SuS	25.3 ± 4 (18-32)	26.7 ± 3.7 (20.2-36.6)	244.1 ± 135 (91-646)
Tjerebukta	TbD	34.6 ± 2.8 (29-40)	20.4 ± 4 (14.5-31)	103.1 ± 73 (31.4-336.3)
	TbL	24 ± 2.4 (19-29)	25.3 ± 4.9 (12.4-33.3)	203.3 ± 113.9 (18.2-433.9)
	TbS	20.7 ± 1.9 (18-24)	21.3 ± 3.1 (13.7-26.2)	114.1 ± 50.8 (23.4-218)
Vastusjärvi	VaD	34.6 ± 2 (31-38)	13.8 ± 2.4 (7.1-19)	19.2 ± 9.3 (2.3-53.9)
	VaL	23.5 ± 2.3 (20-29)	20.5 ± 5.7 (12.3-35.4)	84.9 ± 86.3 (11.8-410.2)
Vuoddasjavri	VdD	37.8 ± 2.6 (26-42)	13.3 ± 2.2 (9.3-22.1)	24.3 ± 15.8 (7.4-111)
	VdL	24.1 ± 2.8 (18-31)	19.5 ± 3.6 (10.5-27.2)	80.3 ± 43.6 (9.3-238)
	VdS	21.9 ± 2.2 (17-27)	20.8 ± 3.9 (10.2-33.3)	105.9 ± 75.9 (8.2-423)
Vuolgamasjavri	VgD	37.2 ± 2.8 (31-41)	22.4 ± 2.7 (17.5-27.7)	135.5 ± 49.8 (62.1-259)
	VgL	25.3 ± 1.8 (20-30)	23.6 ± 5.7 (11.5-38.5)	184 ± 150.6 (17-744)
	VgS	23.5 ± 2.7 (19-30)	22 ± 4.5 (15.5-35.2)	134 ± 106.2 (39.1-548)
Virdnejavri	ViD	29.9 ± 3 (27-37)	24.9 ± 3.5 (18.4-30)	181.2 ± 70.7 (68.4-337)
	ViL	24.9 ± 2.4 (19-29)	25.3 ± 3.7 (13-38.8)	197.2 ± 102.9 (25.5-765)
	ViS	21.4 ± 1.4 (20-25)	24.7 ± 1.2 (22.5-27)	157.4 ± 31.4 (117-219)
VuolitNjivlojavri	VnL	25.4 ± 1.5 (22-29)	26.9 ± 4.5 (14.4-36.4)	230.5 ± 114.6 (28.4-590)
VoulitSpielgajavri	VsL	30.5 ± 2.6 (21-36)	25.6 ± 4.5 (12.5-35.2)	187.5 ± 93.1 (15.4-506)
Vuontisjärvi	VuL	28.1 ± 1.7 (24-31)	17.3 ± 4 (7.4-24.2)	45.7 ± 25.9 (2.9-110.5)



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 Paadar 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 Blaifulchen 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 allochrony 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 km² with a maximum depth of 30 m, and Suohpatjávri covers 2 km² 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

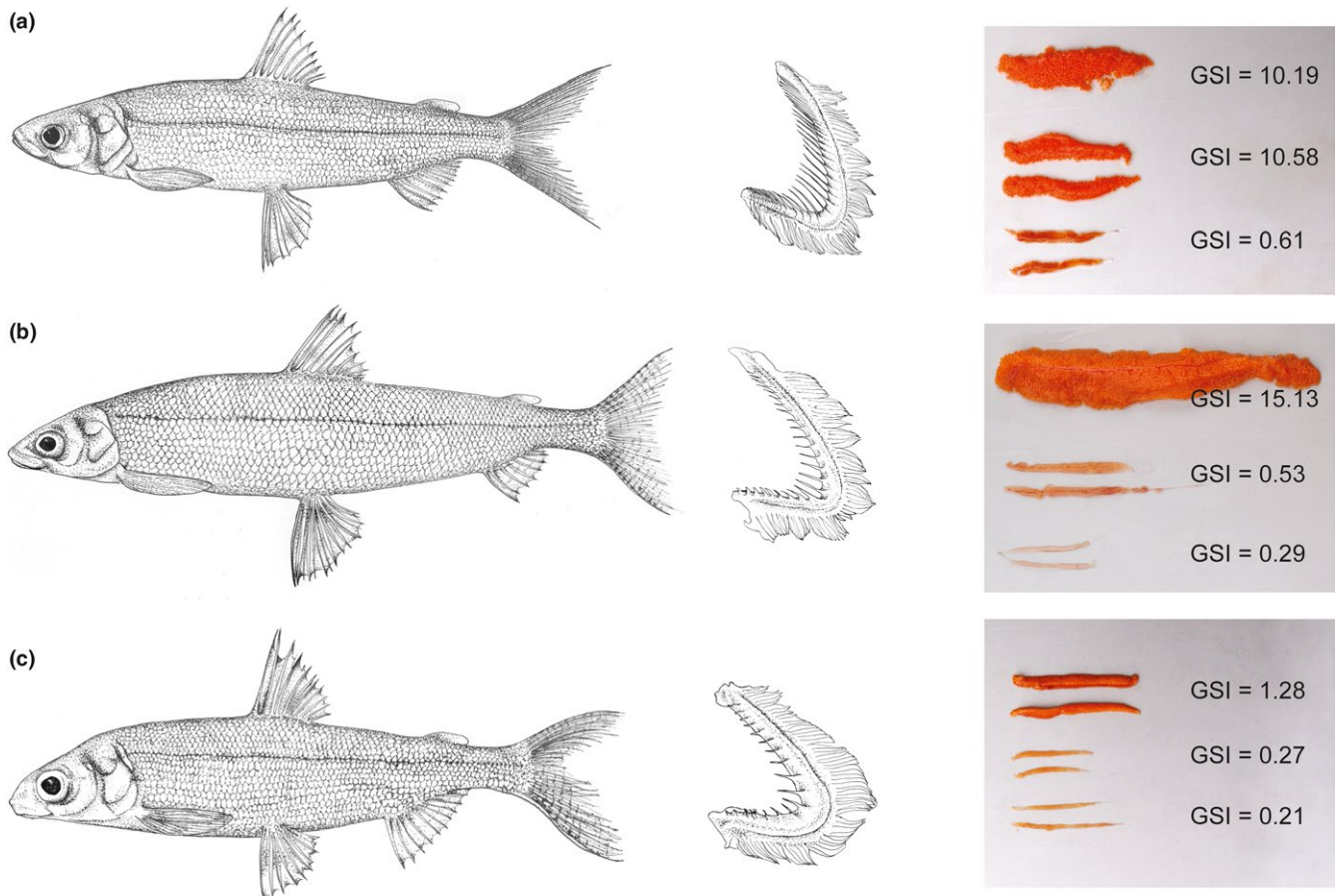


FIGURE 1 Drawings of the three ecomorphs of European whitefish (*Coregonus lavaretus* L.) from northern Norway and their respective gill rakers: (a) DR, densely rakered, (b) LSR, large sparsely rakered, and (c) SSR, small sparsely rakered ecomorph (line drawings modified from Harrod et al., 2010). The 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) ^b	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 ^c	356 ^c	422 ^c	3.12 ^c	50% ^c	0.74 ^c

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

^aBoth 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.

^bOutlier males were removed from the data set and are therefore not included here. A total of 139 mature males. ^cOnly 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°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* (Stuorajävi, DR whitefish), and *BWF1*, *Clatet06* and *Coc1_lav10* (Stuorajävi, 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

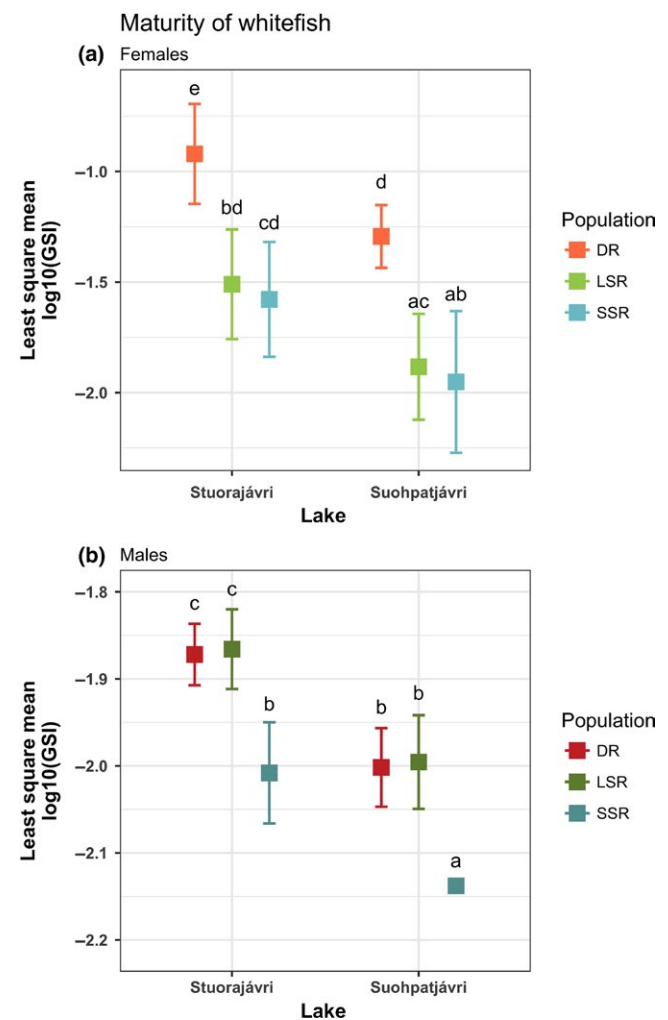


FIGURE 2 Plots showing the differences in maturity of the whitefish ecomorphs, for females (a) and males (b) and across two lakes. This was done using least square means for the specified factors in the linear model, and p -values were adjusted for multiple comparisons by Tukey's HSD. Boxes indicate 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 α 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 km²) and a little deeper (max depth of 30 m) compared with Suohpatjávri (2.0 km², 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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