

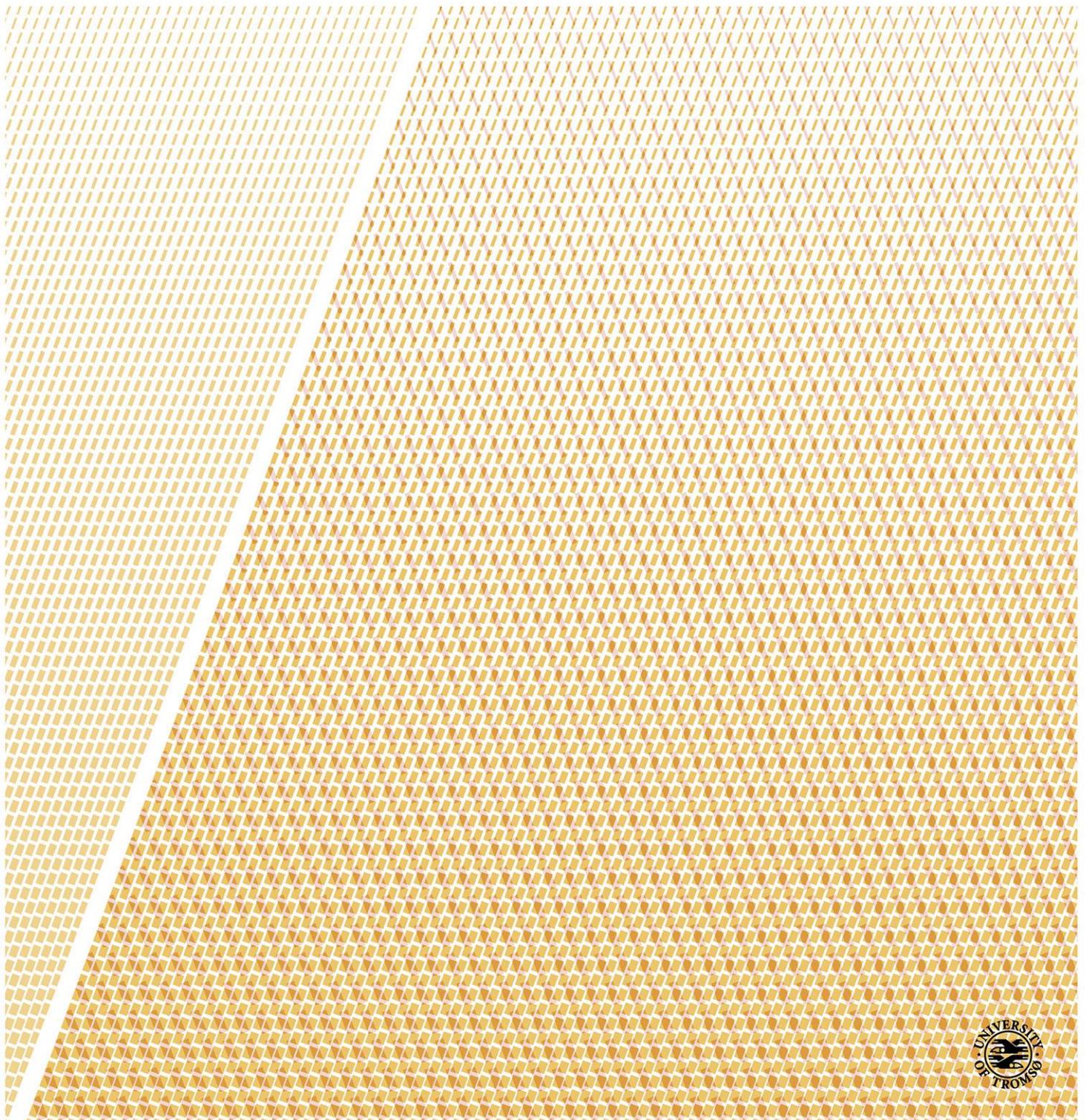
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**Speciation reversal in sympatric eco-morphs of European whitefish
(*Coregonus lavaretus*. L) – phenotypic and genomic consequences**

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SUMMARY

Anthropogenic activities, such as introduction of non-native species, is considered as one of the most significant causes for declining biodiversity, since such introduction may destabilise ecological barriers to gene flow between native populations. In the 1960s a salmonid fish, vendace, was introduced to the upper parts of Pasvik watercourse and during the early 1990s it invaded the entire watercourse, including the lakes studied in this project/thesis. These lakes harbour an eco-morph pair of European whitefish that has diverged in sympatry in post-glacial times. The vendace, being a competitively superior planktivore to the densely rakered eco-morph, relegated this eco-morph from its native pelagic habitat into the non-native littoral habitat, which is mainly occupied by the large sparsely rakered eco-morph. These observations have inspired this PhD thesis to study the phenotypic, genetic, and genomic consequences of the vendace invasion on native sympatric European whitefish eco-morphs. Using neutral microsatellite markers, the study documented that the introduction and invasion of vendace have induced speciation reversal in the eco-morph pair. The comparison of the number of gill rakers from the pre- and post-invasion periods revealed merging of two peaks of distribution of number of gill rakers following the invasion suggesting reduction in discreteness of phenotypes. Population genomic analyses, using genome-wide coverage of SNP markers obtained by sequencing of restriction site associated DNA libraries (RADseq), revealed an unpredicted outcome of speciation reversal at the genomic and functional phenotypic levels. This was attributed to a change in the selective forces during speciation reversal. The introgression patterns at genomic regions of adaptive importance, showed that speciation reversal have unpredictable consequences on introgression. Overall, this PhD study concludes that anthropogenic activities have wide-ranging and stochastic effects for species undergoing speciation reversal.

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1. LIST OF PAPERS AND AUTHORS CONTRIBUTIONS

Paper I: Shripathi Bhat, Per-Arne Amundsen, Rune Knudsen, Karl Øystein Gjelland, Svein-Erik Fevolden, Louis Bernatchez, Kim Præbel. Speciation reversal in European whitefish (*Coregonus lavaretus* (L.)) caused by competitor invasion. *PLoS ONE* 9:e91208.

Paper II : Shripathi Bhat, Louis Bernatchez, Per-Arne Amundsen, Svein-Erik Fevolden, Laura Benestan, Kim Præbel. Speciation reversal leads to unpredictable genomic outcomes in European whitefish. Manuscript draft.

Paper III: Shripathi Bhat, Fabien C Lamaze, Louis Bernatchez, Kim Præbel. Effect of speciation reversal on genomic introgression in sympatric whitefish eco-morphs. Manuscript draft.

Contributions (alphabetical order)

	Paper I	Paper II	Paper III
Concept and idea	KP, PAA, RK	KP, PAA,SB	SB
Study design and methods	KP, PAA,SB	KP, LB, LaBe, SB	FL, SB
Data gathering and interpretation	KØG, KP, PAA, RK, SB	KP, LB, LaBe, PAA, SB	FL,KP, LB, SB
Manuscript preparation	KØG, KP, LB, PAA, RK, SB, SEF	KP, LB, LaBe, PAA, SB, SEF	FL, KP, LB, SB

KP: Kim Præbel, KØG: Karl Øystein Gjelland, LB: Louis Bernatchez, LaBa: Laura Benestan, PAA: Per-Arne Amundsen, RK: Rune Knudsen, S-EF: Svein-Erik Fevolden, SB: Shripathi Bhat

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



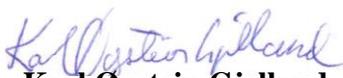


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Svein-Erik Fevolden Laura Benestan Louis Bernatchez Fabien C Lamaze


Karl Øystein Gjelland

2. INTRODUCTION

2.1. Barriers to gene flow

One of the important questions in evolutionary biology is how new species are formed. Speciation is a continuous and ongoing process (Coyne and Orr 2004) and it is defined as the divergence of an ancestral lineage into descendent lineages that are genetically differentiated and reproductively isolated (Nosil et al. 2009b). Genetic differentiation and reproductive isolation between descendent lineages will evolve as barriers to gene flow build-up. Barriers to gene flow are biological features of the diverging populations that impede the exchanges of genes between them (Coyne and Orr 2004). In ecological speciation, a special case of speciation, barriers to gene flow evolve between populations as a result of ecologically based divergent selection (Schluter 1996, Rundle and Nosil 2005, Via 2009).

Barriers to gene flow are classified based on their presence during the life history of the diverging populations (before mating, fertilization and/or after fertilization). Pre-mating isolation barriers are barriers that impede the gene flow before the transfer of gametes to members of other species or populations. They can be intrinsic or extrinsic (Coyne and Orr 2004). Intrinsic pre-mating isolation involves incompatibility in reproductive organs or in mating behaviour (sexual selection) (Seehausen et al. 1997, Boughman 2001), whereas extrinsic factors include variability in time and place of spawning (Svårdson 1979, Vonlanthen et al. 2009, Klemetsen 2010), and selection against immigrants (Nosil et al. 2005), which prevents heterospecific encounters.

Ecological and behavioural inviability forms the extrinsic post-zygotic barriers to gene flow. In ecological inviability, hybrids are less likely to survive in the parental habitats. This is caused by the lack of intermediate niches or reduced feeding efficiency due to non-optimal

feeding apparatus, asynchronous hatching time, and larval emergence (Hatfield and Schluter 1999, Woods et al. 2009), whereas in behavioural inviability fertile intermediate phenotypes render unattractive and fail to obtain mates (Stelkens et al. 2008). Intrinsic post-zygotic isolation barriers include hybrid inviability and hybrid sterility, and are characterised by developmental deformities (causing mortality) and reproduction related defects (Coyne and Orr 2004). These defects may arise due to accumulation of unfavourable allelic combinations leading to Bateson–Dobzhansky–Muller (BDM) incompatibilities (Seehausen et al. 2014). Genomic incompatibilities may trigger the reactivation of transposable elements (Dion-Cote et al. 2014), and mis-expression of genes related to growth in hybrids (Renaut et al 2011). The role of natural selection is well established in the development of reproductive isolation between diverging populations (Nosil 2012). Population level approaches are now gaining popularity in studies of speciation, especially in cases where evolutionarily young eco-morphs undergo divergence fuelled by natural selection. Natural selection enables the interaction between ecology, phenotypes, and genetics. Hence, identifying the loci showing signatures of divergent selection will form a good starting point to unravel mechanisms by which the reproductive isolation forms (“the magnifying glass approach” as suggested by Via (2009)).

2.2. Hybrid zones

Hybrid zones are geographical regions where two genetically distinct populations meet and reproduce. They have been called “natural laboratories” for evolutionary studies (Hewitt 1988) and enable studies of the effects of natural selection on non-parental (hybrid) individuals. Hybrid zones also act as ‘selective filters’, where hybrids effectively filter gene flow by preventing introgression of genomic regions responsible for the reproductive isolation (and/or adaptation) and let other genomic regions introgress freely (Martinsen et al. 2001). Quantification of the strength of reproductive isolation can be done by assessing the

introgression of foreign alleles into non-native genetic backgrounds (Gompert and Buerkle 2009).

2.3. Speciation reversal and the role of anthropogenic activities

“It is becoming clear that human activities have a more profound effect on biodiversity than was previously believed, particularly in the animal kingdom, by inhibiting the process of species divergence within certain ecosystems”- Philip Hunter (2006).

A large part of the world’s species biodiversity is evolutionarily young and has evolved as a by-product of divergent adaptation to heterogeneous environments (Seehausen 2006). Various natural and anthropogenic activities such as climate change, habitat destruction, and introduction of non-native species can destabilise these heterogeneous environments. This will weaken the reproductive barriers between diverging species and ultimately lead to the formation of hybrid swarms. This process has been termed “speciation reversal” or “speciation in reverse” (Coyne and Orr 2004). Exemplary cases of this process have been reported in various study systems such as alpine whitefish (*Coregonus* spp), cichlids (*Pundamilia* spp), ciscoes (*Coregonus* spp), European whitefish (*Coregonus lavaretus*), and three-spined sticklebacks (*Gasterosteus aculeatus*) (Seehausen et al. 2006, Todd and Stedman 1989, Taylor et al. 2006, Vonlanthen et al. 2012, **Paper I**).

2.4. Genomic consequences of hybridisation and introgression

A considerable interest exists in understanding the genetic architecture behind local adaptation and barriers to gene flow (Wu 2001, Coyne and Orr 2004, Hoekstra and Coyne 2007, Vonlanthen et al. 2012). The theory of speciation with gene flow predicts that the genetic difference between the two populations will increase as the process of divergence proceeds (Feder et al. 2012). Many empirical studies have shown that the genome is

semipermeable to gene exchange and hence show variable level of genetic differentiation across the genome (Nosil et al. 2009a, Feder et al. 2012). As suggested by Feder et al. (2012), divergent selection drives genetic differentiation at certain genomic locations across the genome in the initial phase of population divergence (DS). These regions with elevated differentiation are termed “genomic islands of divergence” (Nosil et al. 2009a). As the process of genomic divergence continues, divergent hitchhiking (DH) overtakes direct selection (DS). In the divergent hitchhiking stage, genomic islands grow in number and size as the recombination rate decreases. As the gene flow at unlinked loci is reduced, genome hitchhiking (GH) supersedes the effect of DH and will ultimately result in a reduction of the total genome-wide recombination rate leading into the final post-speciation stage. When two species have reached the final post-speciation stage, they can rarely hybridise, and if they do, it leads to mal-adapted phenotypes in the hybrids. However, when populations that are in early stage of divergence come into secondary contact, it may result in increased hybridisation and introgression, as the genomic divergence has not reached the final post-speciation stage.

Genome-wide analyses of introgression across the animal and plant kingdoms have suggested variable porosity to foreign alleles across the genome (Payseur et al. 2004, Turner et al. 2005). Introgression has been shown to lead to *de novo* chromosomal rearrangements (Lai et al. 2005), to recombination suppression at chromosomes (Ostberg et al. 2013), and meiotic breakdown (Dion-Cote et al. 2015). Interspecific recombination at chromosomes, promoted by hybridisation and introgression, may also facilitate the breakdown of co-adapted gene complexes, resulting in outbreeding depression (Gharrett et al. 1999, Gilk et al. 2004). In contrast, when recombination is suppressed at these co-adapted gene complexes, it may result in heterosis that provide a fitness advantage for the hybrid individuals. With the advancement in understanding of the genomics of speciation and hybridisation, it has been suggested that

some parts of the genome are more resistant (or more prone) to introgression, than others (Gompert and Buerkle 2009, Nolte et al. 2009a, Payseur 2010). In hybrids, the introgression pattern (e.g. neutral, negative and positive introgression) at individual loci is a consequence of the fitness effect of genotype combinations. Hence, contrasting the introgression pattern of markers situated across the genome permits identification of loci or genomic islands that impart local adaptation and/or reproductive isolation (Gompert and Buerkle 2010).

3. THE STUDY SYSTEM

Post-glacial lakes in the Northern hemisphere are relatively young in evolutionary terms (11,000-12,000 ybp) and exist as replicates across the Fennoscandian landscape. This PhD thesis includes samples from Lake Skrukkebukta and Lake Vaggetem situated in the Pasvik watercourse in northern Norway. The study lakes are characterised by low species abundance and many vacant niches enabling adaptive divergence of different eco-morphs. This provides an excellent situation for studies of ecological speciation (Østbye et al. 2006, Klemetsen 2010, Præbel et al. 2013b). European whitefish (*Coregonus lavaretus* L.) is a polymorphic species in Fennoscandia and commonly occurs as sympatric eco-morphs that are characterised by differences in morphology and number of gill rakers (Amundsen et al. 2004, Siwertsson et al. 2010, Siwertsson et al. 2012). The number of gill rakers is associated with trophic specialization and has an important role in the adaptive radiation of coregonids (Bernatchez 2004, Præbel et al. 2013b). It has also been suggested that the number of gill rakers has an additive genetic component (Svärdson 1952, Bernatchez 2004). The pelagic eco-morph (densely rakered whitefish, DR) is a zooplankton feeder and is characterised by long, thin and densely situated gill rakers, whereas the littoral eco-morph (large sparsely rakered whitefish, LSR) is a littoral feeder, having short, thick and sparsely placed gill rakers (Amundsen et al.

2004). Throughout the synopsis, I will use DR eco-morph for DR whitefish and LSR eco-morph for LSR whitefish.

Analyses using two mitochondrial DNA segments, suggested that a single ancestral clade colonized the region from eastern refugia of glacial lakes (Østbye et al. 2005a). Repeated sympatric divergence of the DR eco-morph from the LSR eco-morph has been shown using microsatellite loci and morphological analyses and these analyses revealed a small, but significant, genetic differentiation between them indicating partial reproductive isolation (Østbye et al. 2005b, Østbye et al 2006, Præbel et al. 2013b). In the 1960's vendace (*Coregonus albula*), a salmonid, was intentionally introduced into the tributaries of Lake Inari to enhance the commercial fishery in the lake (Mutenia and Salonen 1992, Præbel et al. 2013a). Lake Inari forms the headwater for the Pasvik watercourse and during the 1990's vendace invaded the whole watercourse including the study lakes (Amundsen et al. 1999). The zooplanktivorous vendace is competitively superior to the DR eco-morph and a rapid increase in population density of vendace have destabilised the whole ecosystem of the study lakes (Bøhn and Amundsen 1998, Bøhn and Amundsen 2001). Ecological studies have documented the competitive relegation of the DR eco-morph from its preferred pelagic habitat into the littoral zone (Amundsen et al. 1999, Bøhn et al. 2008). Based on reports of increased catches of the DR eco-morph in littoral habitat (Amundsen 1999), this study assumes that the habitat relegation of DR eco-morphs has increased the probability of encounters between the DR and LSR eco-morphs.

4. OBJECTIVES

The main objective of this thesis has been to elucidate the phenotypic, genetic, and genomic consequences of the vendace invasion in the Pasvik watercourse on the native sympatric whitefish eco-morphs.

Specific questions raised were:

1. What is the effect of the vendace invasion on the reproductive isolation between sympatric eco-morphs of European whitefish and does it promote “speciation reversal”? (**Paper I**)
2. What are the genome-wide consequences of speciation reversal in European whitefish eco-morphs and are these genomic consequences predictable? (**Paper II**)
3. How does speciation reversal affect introgression at putatively adaptive loci in European whitefish? (**Paper III**)

5. MATERIALS AND BRIEF SUMMARY OF DATA

The samples used for **Paper I** consisted of gill arches from DR and LSR eco-morphs from two sampling years; at the arrival of vendace (1993) and after its invasion and establishment (2008) in Lake Skrukkebukta. In **Paper II** and **Paper III**, samples from Lake Vaggetem collected early/pre- (1993) and post-invasion (2007) were included along with the Lake Skrukkebukta samples. As it is difficult to identify the hybrids of DR and LSR eco-morphs in the field, the individuals were classified into two populations, either DR or LSR eco-morphs, based on the overall gill raker and body morphology following Amundsen et al. (2004). Pure DR and LSR eco-morphs and their hybrids were subsequently identified genetically through Bayesian analyses using the software STRUCTURE v2.3 (Pritchard et al. 2000). In **Paper I**, individuals were genotyped at 16 microsatellite loci for the genetic analysis and the number of

gill rakers was used for the phenotypic analysis to elucidate whether the eco-morphs showed signatures of speciation reversal. In **Paper II**, a subset of the individuals from **Paper I** were genotyped at 6156 SNPs (Single Nucleotide Polymorphisms) obtained from sequencing RAD (Restriction site Associated DNA) libraries in order to identify the signatures of speciation reversal at the genome-wide level. **Paper III** used the same individuals as in **Paper II** and a subset of SNPs that showed high genetic differentiation between the eco-morphs, to elucidate how speciation reversal affects the introgression patterns at these SNPs.

6. SUMMARY OF THE PAPERS

6.1 Speciation reversal in European whitefish eco-morphs (Paper I)

The comparison of gill raker numbers revealed two discrete distributions pre-invasion, whereas the peaks of these distributions had grown closer post-invasion, suggesting a reduction of distinctiveness in phenotypes. Genetic analyses of the pre-invasion samples suggested that two gene pools were present, as compared to only a single gene pool in the post-invasion samples. The association between number of gill rakers and genotypic data were strong and significant in the pre-invasion samples, whereas a weak and non-significant association was observed in the post-invasion samples. Altogether, these findings suggested that the vendace invasion has triggered breakdown of reproductive isolation between the two eco-morphs of whitefish leading to speciation reversal.

6.2 Genomic consequences of speciation reversal (Paper II)

In both study lakes, the genome-wide pattern of genetic differentiation, measured as F_{st} per SNP locus, showed a significant reduction post-invasion. This suggested a weakening of reproductive isolation between the eco-morphs post-invasion. Moreover, the study revealed a

genome-wide increase of heterozygosity and linkage disequilibrium in the putatively adaptive genomic regions post-invasion. This was mainly observed for the DR eco-morph, suggesting a more pronounced genome-wide introgression from LSR into DR eco-morph, than in the other direction. In both study lakes, divergent selection targeted new biological functions and in addition, mostly new SNPs in already existing biological functions post-invasion. The study showed that speciation reversal has genome-wide consequences for the eco-morph pair in both study lakes and that changes in selective forces may mediate unpredictable outcomes of speciation reversal at the functional phenotypic and genomic levels.

6.3 Effect of speciation reversal on the introgression pattern of adaptive genomic regions (Paper III)

We used genomic cline analysis to estimate the introgression patterns of DR alleles into hybrids with LSR eco-morph genetic background at highly differentiated individual SNPs. Within lakes, a significant proportion of SNPs (~70%) that were introgressing non-neutrally prior to speciation reversal were observed to introgress neutrally during speciation reversal. The proportion of SNPs showing non-neutral patterns of introgression were not significantly different between the hybrid zones during speciation reversal. In addition, the number of SNPs influenced by directional selection against homozygous genotypes from the DR eco-morph and over-dominance of heterozygote genotypes, were not different between the hybrid zones. These observations suggest that there is considerable difference in the effects of speciation reversal on the action of evolutionary forces at individual loci in two study lakes. Taken together, the results of Paper III suggest that speciation reversal, induced by an invasion of an ecological competitor, changed the action of selection forces at putatively adaptive loci in unpredictable ways.

7. DISCUSSION

The results of this study will be discussed in relation to factors contributing to reproductive isolation in sympatric whitefish eco-morphs. I will also discuss the consequences of speciation reversal at the genomic level and how the results may provide knowledge for speciation research. Finally, I will deliberate how insights from the genomic studies of speciation reversal may provide more sustainable conservation actions.

7.1. Barriers to gene flow between DR and LSR eco-morphs: what we know, do not know, and predictions from other species

The most important requisite to achieve complete speciation is the establishment of robust reproductive isolation between diverging taxa (Coyne and Orr 2004). Various pre-zygotic, extrinsic and intrinsic post-zygotic barriers to gene flow contribute to the speciation process. In the following section, I will discuss factors that may be important for the reproductive isolation between the DR and LSR eco-morphs to provide insights into possible mechanisms of speciation reversal.

Identifying the barriers to gene flow that drive the formation of eco-morphs in post-glacial fishes is a daunting task, as it requires detailed ecological, physiological, and genomic knowledge. In addition, *in situ* studies of spawning behaviour (e.g. mate choice) and temporal and spatial segregation are needed to complement the other information. *In situ* studies are difficult to conduct in our sub-Arctic and Arctic regions as, for example, whitefish spawn in late autumn or during the winter where unsafe lake ice conditions and extreme temperatures hamper any field activities. Eco-morphs of Arctic charr (*Salvelinus alpinus*) in Lake Fjellfrøsvatn (Troms, northern Norway), have shown strong spatial and temporal segregation in spawning time (Klemetsen 2010), but such information is still lacking for the European

whitefish eco-morphs. Studies of other coregonid species have reported similar segregation in either time and/or place of spawning as the Arctic charr (Svärdson 1965, Bernatchez et al. 1996, Vonlanthen et al. 2009). Unpublished observations from two lakes in Finnmark, northern Norway, suggest that DR and LSR eco-morphs are likely to have partial overlap in breeding seasons but utilize different spawning grounds (Pers. Com. R. Knudsen & K. Præbel). The habitat relegation of the DR eco-morph from its native pelagic zone into the littoral zone are expected to have changed the realized habitat conditions (e.g., water temperature and food resources) for the DR eco-morph. This would subsequently lead to changes in spawning time, increasing the potential for hybridisation (**Paper I**). This may also have affected the development functions, in line with what was observed for the change in functional representation of genes related to development and reproduction in **Paper II**.

The changes in habitat conditions may either induce or impede extrinsic post-zygotic isolation barriers such as ecological inviability. Ecological inviability is attributable mainly to the reduced foraging efficiency due to intermediate gill rakers and/or mouth morphology, or the absence of appropriate ecological niches for hybrids to feed in (Hatfield and Schluter 1999). Although this study found signatures of intermediate gill raker numbers in a few hybrid individuals collected in the period of speciation reversal, a significant proportion of the individuals still carried gill raker numbers within the ranges of the pure parental eco-types (**Paper I**). Adaptive phenotypic traits, such as the number of gill rakers, are likely to be under polygenic control and may show different inheritance patterns than a phenotypic trait under monogenic control (Hatfield, 1997). As a result, it may require more generations of hybridisation to homogenize polygenic phenotypic traits than the 3-4 generations of hybridisation in the study system included herein (**Paper I**). However, the genetic architecture of the number of gill rakers remains to be identified. This allows an alternative hypothesis to

be proposed: the low proportion of hybrid phenotypes, despite the significant number of hybrid genotypes, may suggest strong selection pressure against hybrid phenotypes. This would mean that ecological inviability plays a pivotal role in deciding the fate of hybrids in the study lakes, at least in the early stage of speciation reversal.

Intrinsic reproductive isolation is attained when genomes of the diverging populations accumulate substantial genomic incompatibilities between them. The genetic basis for barriers to gene flow can vary across time, space, and genomes (Harrison and Larson 2014). Genomic incompatibilities have been reported as one of the major drivers of reproductive isolation between the sympatric normal and dwarf lake whitefish eco-morphs (e.g. Renaut et al 2011, Dion-Cote et al. 2014, Dion-Cote et al. 2015). These eco-morphs originate from separate glacial lineages that have accumulated considerable genomic differences before coming into secondary contact in post-glacial times (Bernatchez and Dodson 1990, Bernatchez and Dodson 1991). Genomic incompatibilities may not represent a barrier to gene flow in eco-morphs of European whitefish in northern Fennoscandia as they have a mono-phylogenetic origin and have had relatively short time for divergence (Østbye et al 2005a). On the contrary, the present study did reveal presence of a few genomic regions showing signatures of underdominance, suggesting that genomic incompatibilities may exist between the eco-morphs (**Paper III**). However, genetic drift and other stochastic processes may also show similar introgression patterns, albeit the contribution of genetic drift may be very low in hybrid zones.

7.2. Speciation reversal and genomic divergence

Differences in habitat conditions, metabolism, reproduction, and spatial subdivisions among individuals within populations lead to formation of new species through time. This requires presence of allelic combinations that produce phenotypes with fitness advantages in the new

habitat (Coyne and Orr 2004). Hybrid zones provide an opportunity to predict and study the effect of these new allelic combinations on the fitness of hybrids including the mechanisms promoting reproductive isolation (Nolte et al. 2009, Janousek et al. 2012). Hybrid zones magnify the effects of various evolutionary processes and their interactions (Hewitt 1988), effect of the various forms of natural selection against hybrids (Latour et al. 2014), and allow to test intrinsic genomic incompatibilities (FelClair et al. 1996, Turner and Harr 2014). Some of these interactions may be created in experimental setups, but creating experimental systems that allow for the interaction of processes and forces may be challenging (Kawecki et al. 2012). The systems used in this study represent an excellent example of a natural local hybrid zones, because they have been sampled continuously before, during, and after the invasion of vendace. This will allow us to study the evolutionary and genetic mechanisms underlying divergence and speciation reversal.

The theory of genomic divergence predicts that the genome-wide differentiation increases as divergence progresses (Feder et al. 2012), mainly due to divergent selection on beneficial alleles (e.g. responsible for ecological specialization) and segregation of these alleles into adaptive genomic islands (Stephens et al. 1994, Wu 2001). Herein, signatures of divergent selection were observed at a few loci, which were directly or indirectly involved in local adaptations (**Paper II and Paper III**). When linkage between genomic regions influenced by selection and the nearby surrounding region increases, the size of genomic islands grows, representing the second stage of genomic differentiation, called divergent hitchhiking. At this stage, one should expect a higher proportion of SNPs under divergent selection compared to their proportion at earlier stages of the same speciation process. When speciation reverse, interspecific recombination will hamper alleles to segregate into genomic islands and, thus, counteract local adaptation and divergence. The current study identified a

similar proportion of SNPs under divergent selection before and during speciation reversal, but the SNPs were located in different genomic regions (**Paper II**). Along with this, the study also reported a genome-wide decrease in genetic differentiation and an increase in heterozygosity and linkage disequilibrium at putative adaptive regions upon speciation reversal, indicating possible weakening of DH (**Paper II**). The changes in the existing selective forces after the habitat relegation of the DR eco-morph may have triggered the selection to target new or similar important biological functions and/or new SNPs in already existing biological functions as observed in **Paper II** and **Paper III**.

Studying introgression patterns at genomic regions assists identification of genes important for local adaptation and genes involved in reproductive isolation. As expected these genomic regions show negative and under-dominance introgression respectively (Raufaste et al. 2005, **Paper III**). Possibly new allelic combinations at loci responsible for local adaptation and reproductive isolation may lead to mal-adapted phenotypes in hybrids. This is more evident when populations are far in the speciation process and have accumulated a substantial amount of genomic incompatibilities (Raufaste et al. 2005, Mallet 2006). In contrast to under-dominance, signature of over-dominance type of introgression was also observed in a few markers in the present study (**Paper III**). The presence of over-dominance suggests an adaptive advantage for the hybrids. This leads to another perspective on how hybridisation contributes to maintaining the adaptive radiation and new phenotypes with positive fitness that cannot be reached through gradual processes of evolution in either of the diverging populations (Abbott et al. 2013, Seehausen et al. 2014). In the wake of constantly changing living conditions forced by natural or anthropogenic activities, new genetic variation arising through hybrids may be more important and interesting to study than ever before. This especially holds true for weakly reproductively isolated populations that are associated with

fragile barriers to gene flow. Such populations will experience neutral introgression at once moderately differentiated genomic regions as shown in **Paper III**, suggesting that speciation reversal can reverse the effect of divergent hitchhiking. The population genomic analyses of genomic regions with adaptive importance further highlight the fragility of the reproductive isolation between eco-morphs of post-glacial fishes that are at the early stage of sympatric divergence.

7.3. Speciation reversal studies and its implications on management

Biodiversity is declining at the global scale with serious consequence for the structure and functioning of ecosystems (Butchart et al. 2010, Hooper et al. 2012). Hybridisation and introgression have led to extinction of various taxa in the animal and plant kingdom, pinpointing the importance of understanding these processes (Allendorf et al. 2001). Whitefish are reported as being vulnerable in lakes of the U.K and Central Europe (Kottelat and Freyhof 2007, Winfield et al. 2010), and alpine whitefish populations have been shown to undergo speciation reversal due to industrial eutrophication (Vonlanthen et al. 2012). Similarly, the present study showed that a human induced biological invasion has triggered speciation reversal in several pairs of once genetically differentiated eco-morphs of European whitefish (**Paper I**, **Paper II**, and **Paper III**). Although European whitefish are not regarded as endangered in northern Fennoscandia, the homogenisation of once divergent gene pools across one of the three major watercourses in Finnmark, cannot be desirable.

Traditionally, conservation studies have relied on phenotypic assessment and a handful of neutral genetic markers ranging from allozymes to microsatellites (Allendorf et al. 2001, Allendorf et al. 2010, Arif et al. 2011). Phenotypic information has been used extensively to identify hybrids until a few decades ago (Allendorf et al. 2001). Detection of hybrids using

phenotypic information assumes that hybrids are phenotypically intermediate to parental types. However, as discussed above, it may take several generations to accumulate the phenotypic signal of speciation reversal as shown by the detection of parental phenotypes in genetic hybrids (**Paper I**). Although microsatellites can be used for classification of species and to identify hybrids of coregonid eco-morphs and species (Kahilainen et al. 2011, Præbel et al 2013c, **Paper I**), neutral microsatellite markers will not reveal adaptive and/or genome-wide differences between sympatric morph-pairs, which is a key component in a recent conservation frameworks (Funk et al. 2012). This conservation framework includes adaptive genetic information along with phenotypic information and traditional neutral markers to predict the management status of populations. In conventional conservation genetics, genetic data are mainly used to identify the evolutionary processes such as gene flow and genetic drift, to classify conservation units or estimate the minimum population size, and for population assignments (McMahon et al. 2014). Genomic approaches, such as analyses of thousands of SNPs (Garner et al. 2015, **Paper II**, and **Paper III**), can act as promising means for conservation practices by scaling up the traditional population genetic inferences and by increasing the population genomics resolution (Allendorf et al 2010, Garner et al. 2015, Shafer et al. 2015, **Paper II**).

Identifying traits that contribute to the fitness of populations gives the manager information about how the biotic and abiotic factors should be adjusted within a monitored ecosystem in order to maintain this fitness. Inclusion of markers that reflect local adaptation would also assist in identifying genomic regions that experience the effect of inbreeding depression (Hoffman et al. 2014), and in estimating the effect of a changing environment. The latter was exemplified in the present study as the habitat change of the DR eco-morphs have changed the phenotypic traits targeted by divergent selection (**Paper II and Paper III**). By

considering adaptive genetic information in a conservation and management framework, as the one suggested by Funk et al. 2012, it is possible to prioritise among populations and identify a candidate population (or species) that may cope well with changing climatic and environmental conditions. This information is especially important when establishing refuge populations of endangered species or performing supportive stockings to save declining populations or species (Etheridge et al 2010). By choosing a source population of similar genotype at adaptive loci as the target population, it is possible to avoid outbreeding depression (Moritz 1999, Edmands 2007). However, it is important to maintain the balance between rescuing an endangered population by performing intentional hybridisation and allowing outbreeding depression. Managers can minimize the risk of both, inbreeding and outbreeding, by allowing intentional hybridization only for populations clearly suffering from inbreeding depression by maximizing the genetic and adaptive similarity between the populations.

Conservation strategies have been established for European whitefish and houting (*C. oxyrhynchus*) in e.g. Denmark (Hansen et al. 2008), Germany (Dierking et al. 2014), and UK (Adams et al 2016), but to my knowledge none of these strategies includes the implementation of adaptive loci or genome-wide scans. Hence, the genomic information obtained in this study and the unpredictable genomic outcomes of hybridisation (**Paper II and Paper III**), can be used in framing the conservation strategies in the future.

8. CONCLUDING REMARKS

This study explored the genome-wide consequences of speciation reversal, by comparing the unique set of samples collected within the same systems during divergence and speciation

reversal. The results of this study showed that the process of divergence can be reversed, especially when populations are weakly reproductively isolated. However, the consequences of such a reversal are highly unpredictable at phenotypic and genomic levels. Hence, the unpredictability associated with the outcomes of speciation reversal may further stress the importance of system specific conservation strategies and need for the inclusion of information on adaptive loci and various ecological factors in conservation frameworks.

9. FUTURE STUDIES

Investigating the number of loci shaping the adaptive phenotypes in European whitefish eco-morphs and their mechanisms of action may help in understanding the genetic basis of adaptive phenotypic traits and possibly reproductive isolation. This can be done by associating the genotypes at SNP loci to phenotypic traits from wild hybrids through a mapping technique called “admixture mapping”. The admixture mapping can also be used in conjunction with other quantitative genetic tools (such as additive genetic variance and covariance matrix) to test the role and fate of hybrids in speciation.

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