

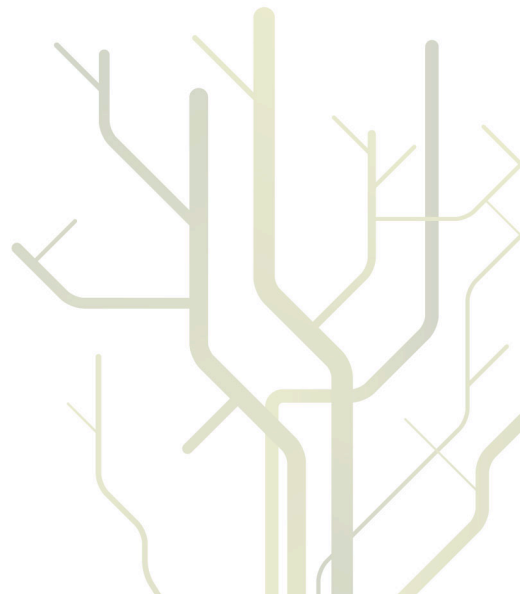
Diversity along a speciation continuum - Ecology and morphology of northern European whitefish (*Coregonus lavaretus*)



Anna Siwertsson

A dissertation for the degree of
Philosophiae Doctor

May 2012



Acknowledgements

There are many people to thank for their contributions, help and support during the work with this thesis. I would first like to thank my excellent team of supervisors: Rune Knudsen, Per-Arne Amundsen, and Colin Adams, for guidance, constructive discussions and for always having confidence in me. Thank you Rune, for your encouragement and “everything is possible” mentality. Thanks Per-Arne, for guiding me during an impressive part of my way to become a Philosophiae Doctor, and also for your more down-to-earth, reality kind of mind. You two make the perfect couple. Thank you Colin, for stimulating scientific discussions and for asking: “what was the question?” I am also grateful for your hospitality during my stay at the Scottish Centre for Ecology and the Natural Environments at Rowardennan, at the shores of Loch Lomond.

This PhD work has included materials sampled during a long period of time, and involved very many people. Thanks to all of you! Especially I would like to thank the fieldwork core team: Rune Knudsen, Per-Arne Amundsen, Kim Præbel, Laina Dalsbø, and Cesilie Lien. All those early mornings and late nights in a small boat under the big skies in Finnmark would not have been them same without you! I would especially like to thank you Kim for not being an ecologist, for your enthusiasm and your willingness to help. Also thanks to Laina, Cesilie, and many others who have helped in fish processing and data collection in the lab. Special thanks go to Jason Newton who kindly taught me the skills of preparing and analyzing samples for stable isotope ratios at the NERC Life Sciences Mass Spectrometry Facility, SUERC, in the outskirts of Glasgow.

I am grateful to Kimmo Kahilainen for sharing his fascination for and knowledge about northern freshwater ecosystems and whitefish morphs. Thanks also for your help with invertebrate sampling and for teaching me the techniques of “mud wrestling”. I would also like to thank Daniel Bolnick for inspiring discussions and refreshing perspectives when hosting me in his lab at the University of Texas at Austin.

“ Science is a social endeavor”, and I am happy to be part of the Freshwater Ecology Group at the Department of Arctic and Marine Biology. Thanks to you all, and to people in the coffee corner for creating such a pleasant working environment. I would especially like to thank Elina Halttunen for being the perfect office mate. We have had many inspiring discussions, and shared frustrations and laughs.

Finally, I would like to thank family and friends for keeping my mind off work now and then. To my parents who sparked my interest for nature and wildlife, and introduced me to the High North: Thank you for all your support and for always being there. Last, but not least, I am so grateful to my little family, Tom Rune and Alvin. Thank you for your love, endless patience and support.

Anna Siwertsson – Tromsø, 30th May 2012

Table of contents

Summary	1
List of papers	3
Introduction	5
Natural selection and ecological speciation	5
The speciation continuum	6
Ecology and progress of speciation	7
The study system - whitefish in northern Fennoscandia	9
Objectives	13
Materials and methods	14
Summary of papers	16
Paper I	16
Paper II	18
Paper III and IV	20
Discussion	22
Parallel adaptive divergence	22
Predicting the level of diversity	24
Stages along a speciation continuum	25
Whitefish – a case of ecological speciation?	28
Closing remarks	29
Conclusions	31
References	32
Original papers	

Summary

How new species are formed is still a major question in evolutionary biology. In ecological speciation, natural selection drives the evolution of slight distinctions between individuals into extensive differences between species. In this thesis, early stages of an ecologically driven speciation process are addressed using ecological and morphological data from a large number of European whitefish (*Coregonus lavaretus*) populations in northern Fennoscandia.

Northern Fennoscandia is an area scattered with lakes created during the glacial retreat (~10-12 kyr BP). Many of these lakes are inhabited by one or up to three different morphs of whitefish. As many other temperate freshwater fishes, whitefish typically diverge into pelagic and littoral resource specialists, the densely rakered (DR) and the large sparsely rakered (LSR) morph, respectively. Parallel within-lake divergence is the most probable origin of these sympatric morphs. In addition, a third whitefish morph (the small sparsely rakered; SSR), specializing on profundal resources, was recently described in a few lakes in the area. The aims of this thesis were to explore and document the phenotypic diversity of whitefish in northern Fennoscandia, and to investigate some of the factors limiting the level of diversity reached within each lake. Further, the ability of LSR whitefish morphs to utilize profundal resources was explored, as this is a probable origin of profundal specialist SSR morphs.

Based on the distribution of gill raker number, a temporally stable morphological trait in the studied populations, I have documented a continuum of increasing diversity in northern Fennoscandian whitefish. This whitefish diversity comprises mono (only LSR), di- (LSR and DR), and tri-morphic (LSR, DR and SSR) populations, in addition to intermediate stages between them. Within three different lakes with dimorphic whitefish populations, some individuals of the LSR morph were specialized to exploit profundal resources. This could indicate an incipient evolution towards a profundal specialist morph (SSR) in some dimorphic lakes. Ecological opportunity (lake size and productivity) is likely a prerequisite for whitefish to diverge, although limitations related to the colonization history also were present.

In conclusion, there is astonishing parallel divergence in ecology, morphology and genetics along a continuum of increasing diversity in whitefish. Thus, ecologically-based natural selection is likely driving the divergence, promoting reproductive isolation and incipient ecological speciation in northern Fennoscandian whitefish populations.

List of papers

The thesis is based on the following papers, which are referred to by their Roman numerals.

- I **Siwertsson, A.**, Knudsen, R. and Amundsen, P.-A. 2012. Temporal stability in gill raker numbers of subarctic European whitefish populations. *Advances in Limnology*. 63: 229-240.
- II **Siwertsson, A.**, Knudsen, R., Kahilainen, K.K., Præbel, K., Primicerio, R. and Amundsen, P.-A. 2010. Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish. *Evolutionary Ecology Research*. 12: 929-947.
- III **Siwertsson, A.**, Knudsen, R., Præbel, K., R., Adams, C.E., Newton, J. and Amundsen, P.-A. *in review*. Discrete foraging niches promote ecological, phenotypic and genetic divergence in sympatric whitefish (*Coregonus lavaretus*). *Evolutionary Ecology*.
- IV **Siwertsson, A.**, Knudsen, R., Adams, C.E. and Amundsen, P.-A. Replicated morphological divergence supports incipient ecological morph formation in European whitefish. Manuscript.

Paper I is reprinted with the permission of Schweizerbart publishers.

Introduction

Natural selection and ecological speciation

The variation of life is astonishing! The best recent estimates suggest that there are between 3 and 10 million different species living on our planet today (Dobson *et al.*, 2008; May, 2011; Mora *et al.*, 2011), and that these represent only ~ 2-4 % of the total number of species that have ever existed (May, 1994). Within all these species, no two individuals are exactly the same. This variation between individuals is the raw material for natural selection and the source of biological diversity. Natural selection is generally recognized as a central mechanism creating local adaptation and phenotypic diversity within species (Endler, 1986; Kingsolver *et al.*, 2001). However, its contribution to the evolution of reproductive isolation has been debated over many decades (Coyne and Orr, 2004), but has now gained general support (e.g. Rundle and Nosil, 2005; Funk *et al.*, 2006; Rueffler *et al.*, 2006; Schluter, 2009). How new species are formed, i.e. speciation, still qualifies for being “that mystery of mysteries” (Darwin, 1859) as it is still one of the major questions in evolutionary biology. There are also alternative causes of speciation not directly associated to natural selection, such as sexual selection, drift, polyploidy and hybridization (Coyne and Orr, 2004). Based on the idea that the mechanisms responsible for divergence within populations possibly also are important for large-scale macroevolutionary phenomena of speciation, we may get important insight to the process of speciation by studying examples of divergence that have not yet reached the level of separate species. This is the background for my thesis where I am focusing on the population-level ecological and morphological differentiation within a polymorphic species and not explicitly on the evolution of reproductive isolation. However, as the research is conducted within the theory of ecological speciation (Schluter, 2000; Schluter, 2001; Rundle and Nosil, 2005; Nosil, 2012), I will give a theoretical background on speciation related to ecological diversity.

Ecological speciation is “the process by which barriers to gene flow evolve between populations as a result of ecologically-based divergent selection” (Rundle and Nosil, 2005). Generally, ecological speciation has three essential components: a source of divergent natural selection, a form of reproductive isolation, and a genetic mechanism linking selection to reproductive isolation (reviewed in Bolnick and Fitzpatrick, 2007; Nosil, 2012). Divergent natural selection may arise from differences between environments or from frequency dependent ecological interactions such as competition and predation (Schluter, 2000; Nosil, 2012). The selection leads to local adaptations in phenotypically diverging groups, and reduced fitness of intermediate hybrids, which represents a reproductive barrier in itself (Schluter, 2001, 2009). Such extrinsic postmating

isolation is therefore likely an important component of ecological speciation (Hatfield and Schluter, 1999; Rundle, 2002; Nosil, 2012). However, alone it may be insufficient for separate species to evolve since hybrids continue to be produced during random mating. Ecological speciation theory therefore usually involves the evolution of assortative mating or any kind of reproductive barrier, e.g. sexual isolation or genetic incompatibilities in hybrids, as a by-product of ecological divergence (e.g. Coyne and Orr, 2004; Nosil, 2012). Additionally, the ecologically based selection against intermediate hybrids may drive the evolution of assortative mating through reinforcement (Servedio and Noor, 2003). The easiest route to speciation by divergent selection in theoretical models is when the trait undergoing adaptive divergence also is causing reproductive isolation, so called magic traits. While some say magic traits are biologically unrealistic (Coyne and Orr, 2004; Bolnick and Fitzpatrick, 2007), others suggest they are not improbable in nature (Nosil and Schluter, 2011; Servedio *et al.*, 2011; McPhee *et al.*, 2012).

The speciation continuum

“Natural selection, also, leads to divergence of character... ...Thus the small differences distinguishing varieties of the same species, will steadily tend to increase till they come to equal the greater differences between species of the same genus, or even of distinct genera.” (Darwin, 1859)

The continuous nature of divergence was noticed and appreciated already by Darwin (1859) and has gained recent interest following the development of the ecological theory of adaptive radiation (Schluter, 2000; Coyne and Orr, 2004; Dieckmann *et al.*, 2004) and the recent focus on ecological speciation (Schluter, 2001; Rundle and Nosil, 2005; Schluter, 2009). Natural populations often vary in their degree of divergence and completeness of reproductive isolation. This variation can be arranged along a speciation continuum of increasingly discrete divergence, from small-scale inter-individual variation in panmictic populations, to ecotypes and discrete polymorphisms within species, and finally to completely reproductively isolated species (Smith and Skúlason, 1996; Hendry, 2009; Nosil *et al.*, 2009; Seehausen, 2009). It has been argued that speciation is often a continuous process with gradually increasing divergence between populations (Mallet *et al.*, 2007; Hendry, 2009; Nosil *et al.*, 2009), and a number of recent studies support this view (Seehausen *et al.*, 2008b; Berner *et al.*, 2009; Peccoud *et al.*, 2009). However, some models and data also indicate that populations may “get stuck” at intermediate states or stages in the speciation process (Bolnick and Fitzpatrick, 2007; Hendry *et al.*, 2009; Bolnick, 2011). Hendry (2009) suggested four states along the continuum of ecological speciation to ease discussions about important factors influencing transitions between them: 1) continuous

variation without reproductive isolation, where individuals at the extremes may be specializing on different resources, 2) discontinuous adaptive variation with minor reproductive isolation, 3) discontinuous variation with strong, but reversible, reproductive isolation, and 4) complete and irreversible reproductive isolation (for details see Hendry, 2009). Populations may remain in one state or move in any direction between the described states, and progress along the continuum is by no means inevitable. Divergent natural selection will not always lead to genetic divergence between populations (Crispo *et al.*, 2006; Bolnick, 2011), and alternative responses to selection includes the evolution of sexual dimorphism and phenotypic plasticity (Rueffler *et al.*, 2006; Bolnick and Fitzpatrick, 2007). In some cases distinct species may even collapse to a hybrid swarm, i.e. “speciation reversal” (Taylor *et al.*, 2006; Seehausen *et al.*, 2008a; Vonlanthen *et al.*, 2012).

When parallel patterns of divergence, i.e. the evolution of similar phenotypes in independent lineages under similar selective regimes, are observed, chance is generally ruled out as an explanation for the observed diversity. Instead, divergent natural selection is likely the most important agent generating such parallel evolution (Endler, 1986; Schluter, 2000). Also, studies of natural replicates of adaptive divergence within species that vary in their progress along the speciation continuum may increase our understanding of factors promoting and constraining the progress of ecological speciation (e.g. Berner *et al.*, 2009; Seehausen, 2009; Rosenblum and Harmon, 2011; Kaeuffer *et al.*, 2012). A multitude of factors have been suggested to be important for the progress of speciation and thus explaining differences between replicate cases of divergent evolution.

Ecology and progress of speciation

“Natural selection, also, leads to divergence of character;... ...for more living beings can be supported on the same area the more they diverge in structure, habits, and constitution...” (Darwin 1859)

Several factors may influence the progress of ecological speciation, and I have broadly divided them into ecological and non-ecological factors. The characteristics of biotic and abiotic environmental factors will determine the strength of divergent selection acting on phenotypic evolution and speciation. Larger differences between environments will generally generate stronger selection, and also selection acting on multiple traits and ecological dimensions, and promote evolutionary divergence and speciation (Nosil *et al.*, 2009; Nosil, 2012). Divergent selection may also arise from frequency-dependent competition among conspecifics for a limited resource in combination with

ecological opportunity (e.g. Schluter, 2000; Dieckmann *et al.*, 2004). Frequency-dependence means that more similar individuals will experience stronger selection and thus an individual's fitness is dependent on the frequencies of phenotypes in the population (Schluter, 2000). Also other ecological interactions that are frequency-dependent, e.g. predation, may be important in a speciation process (Nosil and Crespi, 2006; Langerhans *et al.*, 2007; Meyer and Kassen, 2007). Ecological opportunity loosely refers to the wealth of resources and absence of interspecific competitors (Schluter, 2000), and is expected to facilitate population divergence and increase the number of new types formed in an evolutionary divergence process (Losos and Schluter, 2000; Schluter, 2000; Seehausen, 2006a), but see (Losos, 2010). In addition to generating divergent selection, ecological factors are also important for enabling stable coexistence of competing populations, and allowing persistence of populations colonizing new environments. In order to coexist, populations commonly diverge along diet or habitat axes (Robinson and Wilson, 1994; Schluter, 1996). Often the same ecological conditions that allow coexistence also generate the divergent selection, and the temporal stability of these conditions is important for the evolution of substantial reproductive isolation (Nosil, 2012). In variable environments evolution of phenotypic plasticity is generally favored over genetic divergence and speciation (West-Eberhard, 1989; Price *et al.*, 2003; West-Eberhard, 2005; Pigliucci *et al.*, 2006; Pfennig *et al.*, 2010; Fitzpatrick, 2012). Altogether, a multitude of ecological factors may be important for the evolution of population divergence and speciation, and the strength of divergent selection may vary with e.g. population density, resource availability, predator regimes, and interspecific competition (Svanbäck and Persson, 2009; Bolnick, 2011; Kaeuffer *et al.*, 2012).

A number of non-ecological factors are also important for population divergence and ecological speciation. Speciation is generally promoted by physical distance and geographic barriers that reduce gene flow between diverging populations (Coyne and Orr, 2004). Longer time since the start of the divergence process, which is related to a larger number of generations, will also tend to increase the level of reproductive isolation between populations (Coyne and Orr, 1989; Coyne and Orr, 2004). The specific mating system within the study organism will influence the possibility of different kinds of reproductive barriers to evolve (Hendry, 2009). For example, the evolution of mate choice seems to be important in several cases of ecological speciation in animals (Rundle *et al.*, 2000; Nosil *et al.*, 2002; Langerhans *et al.*, 2007; Grant and Grant, 2008; Seehausen, 2009; Tobler *et al.*, 2009; Merrill *et al.*, 2011). In addition, a number of genetic factors, such as standing genetic variation and the architecture of ecological and reproductive traits under selection, may differ between groups and will affect the response to selection (Seehausen, 2004; Barrett and Schluter, 2008; Schluter and Conte, 2009; Berner *et al.*, 2010).

The interest in ecological speciation has led to an increasing number of model systems being described (Hendry, 2009). A large number of case studies, from various disciplines and taxonomic groups, are indeed needed to be able to draw general conclusions about mechanisms important in the process of ecological speciation (Gavrilets and Losos, 2009). Fishes in postglacial lakes have become popular model systems for the study of adaptive diversification and ecological speciation as several taxa are in the early stages of the process and occur in multiple lakes which provides a promising scenario for comparative studies (Schluter, 1996; Rundle *et al.*, 2000; Schluter, 2000; Bernatchez, 2004; Østbye *et al.*, 2006; Bernatchez *et al.*, 2010).

The study system - whitefish in northern Fennoscandia

Coregonids on the northern hemisphere appears to be good natural model systems for ecological speciation because adaptive divergence and reproductive isolation have evolved in postglacial time (Bernatchez, 2004; Østbye *et al.*, 2005a; Østbye *et al.*, 2006; Vonlanthen *et al.*, 2009; Bernatchez *et al.*, 2010; Hudson *et al.*, 2011). European whitefish (*Coregonus lavaretus*) is a highly polymorphic species with up to five different forms coexisting in the same lake (Svärdson, 1979; Hudson *et al.*, 2011). Sympatric forms within lakes are usually called “morphs”, even though they generally are reproductively isolated (Østbye *et al.*, 2005b; Østbye *et al.*, 2006; Hudson *et al.*, 2011; Præbel *et al.*, *in press*; Præbel *et al.*, *submitted*).

Whitefish morphs are traditionally named and identified by differences in morphology and number of gill rakers (e.g. Svärdson, 1952, 1957, 1979; Amundsen, 1988b; Himberg and Lehtonen, 1995), which also correlates with other traits in head and body morphology (Kahilainen and Østbye, 2006; Harrod *et al.*, 2010). Despite the lack of experiments estimating genetic and plastic components to gill raker number variation in European whitefish, a high additive genetic component is suggested based on heritability estimates of gill raker numbers in Lake whitefish (*C. clupeaformis*) (Bernatchez, 2004) and other fish species (e.g. Day *et al.*, 1994; Hatfield, 1997; Foote *et al.*, 1999; Funk *et al.*, 2005). In addition, artificially produced hybrids between European whitefish morphs express intermediate gill raker counts (Svärdson, 1952). However, plastic responses in gill raker number have been suggested (Lindsey, 1981) and observed in experimental conditions (Todd, 1998), although with small effects in absolute number.

Whitefish, like other northern temperate fishes, colonized available freshwater habitats in northern Fennoscandia following the glacial retreat (~10-12 kyr BP).

Phylogeographic studies using mitochondrial DNA indicate that a single ancestral clade colonized the whole region, probably originating from an eastern refugium of glacial lakes (Østbye *et al.*, 2005b; Hudson *et al.*, 2011). As in several other postglacial fish species (reviewed in: Schluter and McPhail, 1993; Robinson and Wilson, 1994), the most common co-occurring whitefish morphs in these lakes specialize on pelagic and littoral resources, respectively (Amundsen, 1988b; Amundsen *et al.*, 2004a; Amundsen *et al.*, 2004b; Østbye *et al.*, 2006). The morphs are usually named according to the characteristics of their gill raker apparatus. The pelagic whitefish morph (referred to as the densely rakered morph, DR; Kahilainen *et al.*, 2004) is a zooplanktivore specialist and has many, long and densely packed gill rakers (Fig 1) (Amundsen *et al.*, 2004b). The littoral morph (referred to as the large sparsely rakered morph, LSR; Kahilainen *et al.*, 2004) has shorter, fewer, and more widely spaced gill rakers (Fig 1). The LSR morph is considered to be a more generalist forager, but specializes on benthic macro-invertebrates when coexisting with the DR morph (Amundsen *et al.*, 2004b). A high number of densely packed gill rakers is expected to facilitate the retention of small-sized zooplankton prey (Sanderson *et al.*, 2001), while larger inter-raker distances would be more beneficial for benthic foraging fish, e.g. by preventing bottom sediment from clogging the gill raker apparatus. The close association between resource use and morphological traits in whitefish morphs (Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010) indicate an adaptive value of the observed morphological differences. In addition, indirect evidence comes from individual level correlations of gill raker number and zooplankton prey size within and between the different morphs (Kahilainen and Østbye, 2006; Kahilainen *et al.*, 2011), suggesting a functional importance in food acquisition.

Sympatric DR and LSR whitefish morphs occur in numerous lakes in northern Fennoscandia, and express astonishing parallels in both ecological (diet, habitat, life history), morphological (gill raker number), and genetic divergence (Østbye *et al.*, 2006). This replicated evolution suggests that divergent natural selection is important for the differentiation of the DR and LSR morphs. Divergent natural selection may be important both for driving ecological and evolutionary divergence and for maintaining already existing differences between morphs. However, genetic analyses have suggested that within-lake postglacial divergence is the most probable origin of the DR and LSR morphs in northern Fennoscandian lakes (Østbye *et al.*, 2005a; Østbye *et al.*, 2006), thus supporting a sympatric ecological speciation scenario.

More recently, a third whitefish morph, the small sparsely rakered (SSR; Kahilainen *et al.*, 2004), was found to coexist with DR and LSR morphs in a few

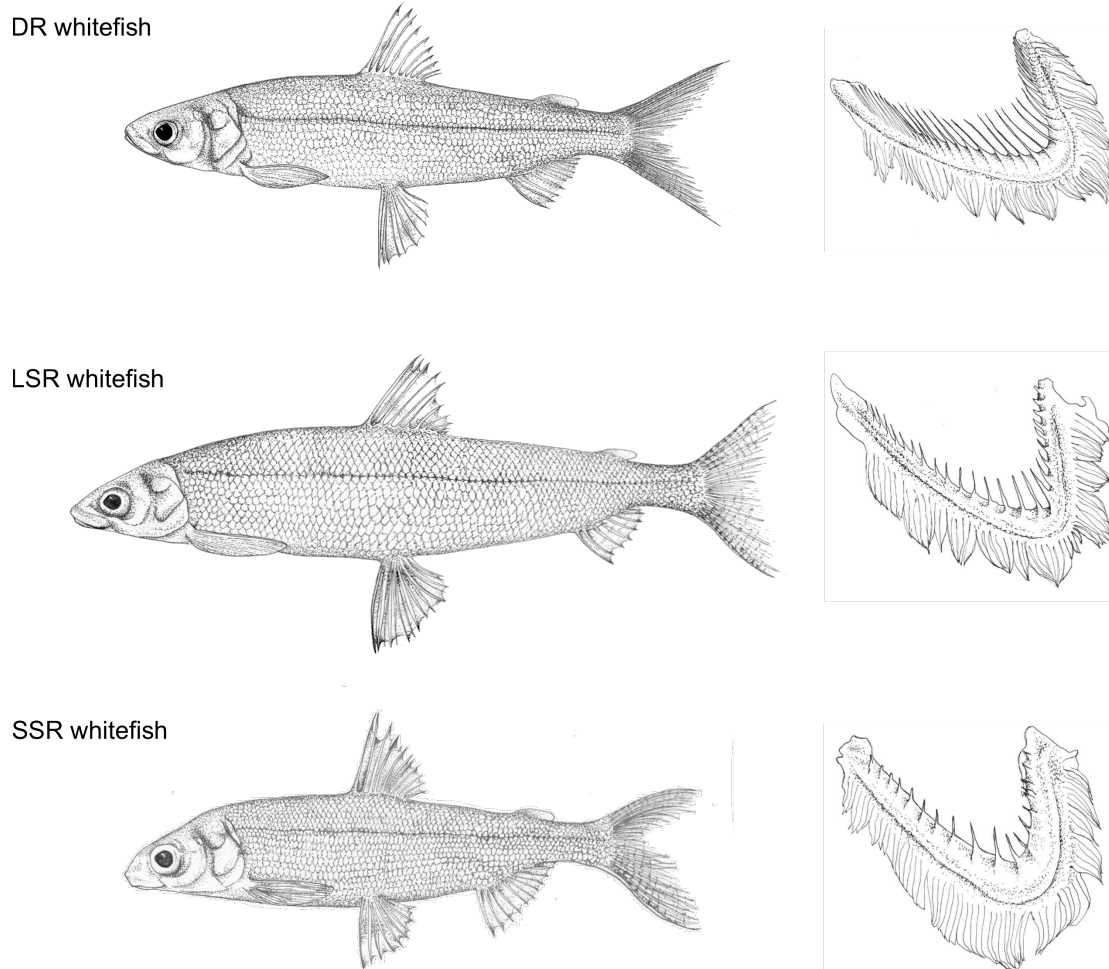


Figure 1. The three whitefish morphs, and their gill raker characteristics, present in northern Fennoscandian lakes: densely rakered (DR), large sparsely rakered (LSR), and small sparsely rakered (SSR) whitefish. Modified from Harrod *et al.* (2010).

lakes in the Pasvik/Paatsjohki watercourse (Kahilainen *et al.*, 2004; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010; Kahilainen *et al.*, 2011; Præbel *et al.*, *submitted*). The SSR whitefish morph has very few gill rakers (Fig 1), and is ecologically specialized to utilize profundal resources (Kahilainen *et al.*, 2004; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010; Præbel *et al.*, *submitted*). Divergence and specialization to the profundal niche is less studied, and likely also less frequent, than the common pelagic-littoral dichotomy in temperate freshwater fishes (but see: Turgeon *et al.*, 1999; Zimmerman *et al.*, 2006; Klemetsen, 2010).

The replicated occurrence of allopatric and sympatric whitefish morphs in numerous lakes, and the diversification beyond the common pelagic-littoral dichotomy are exciting features of the northern Fennoscandian whitefish. Thus, they constitute a promising natural model system for further exploration of adaptive divergence and ecological speciation. This thesis addresses the early

stages of an ecologically driven speciation process by exploring the variation of whitefish diversity in ecology and morphology. I investigate some factors that may limit the level of diversity reached by whitefish in different lakes and in particular address an incipient divergence to exploit profundal resources. The research is based on extensive sampling of whitefish covering 39 lakes in three watercourses and analyses of stomach contents, stable isotope ratios, morphology, and genetics.

Objectives

The theory of ecological speciation constitutes the background for this thesis, and I have mainly focused on the importance of ecologically-based divergent selection in generating the observed patterns of diversity. Even though I refer to the divergence process as a speciation process, I am not actually looking into mechanisms of reproductive isolation (but see Discussion). The overall aim of the thesis is to study variation in whitefish ecology and morphology in relation to a speciation continuum. Specific objectives were to:

1. Explore the temporal stability of gill raker numbers in order to evaluate the reliability of this trait for identifying adaptive divergence in northern Fennoscandian whitefish populations (Paper I)
2. Explore the variability in whitefish phenotypic diversity (using gill raker number) covering a large geographic area in northern Fennoscandia (Paper II)
3. Explore possible promoting and constraining factors that affect the level of phenotypic diversity reached by whitefish in different lakes (Paper II)
4. Explore a possible incipient divergence within the LSR whitefish morph to exploit profundal resources, i.e. a divergence beyond the pelagic-littoral dichotomy (Paper III, IV)

Materials and methods

This thesis is based on extensive sampling of whitefish covering 39 different lakes in northern Fennoscandia (Fig 2). The studies within this thesis have been conducted on different time and geographic scales (Table 1). For methodical details of the specific studies, I refer to the respective papers. However, common for all studies is the method for identifying the different whitefish morphs. The morph affiliation of each individual whitefish was classified in the field, by a limited number of people, according to appearance, head and body form and a visual evaluation of the gill raker morphology (Fig 1) (Amundsen, 1988b; Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010). SSR whitefish is identified by large eyes, large head, pronounced subterminal mouth, reddish fins and extremely short and widely spaced gill rakers. LSR whitefish is usually larger in size with typical whitefish coloration with silvery sides, dark back and fins and robust gill rakers with intermediate length and spacing. The DR whitefish is usually smaller sized, silvery and has long, thin and densely packed gill rakers. In the laboratory the number of gill rakers on the first left branchial arch was counted under a dissecting microscope, and these counts were used to confirm the *a priori* morph assignments performed in the field. This method of classifying whitefish morphs “by eye” has been evaluated in a population with sympatric DR and LSR whitefish morphs (Amundsen *et al.*, 2004a), and a 98 % classification success was documented when comparing *a priori* classifications with gill raker morphometric data (length, breadth, spacing). When they added gill raker number to the multivariate discrimination function, the classification success increased to 100 %. Thus, the *a priori* classification of whitefish morphs in northern Fennoscandian lakes appear to be reliable, and we prefer to use this method over defining the morphs solely based on gill raker counts.

Table 1. The number of watercourses, lakes and years for each lake included in the different studies.

	Watercourses	Lakes	Years
Paper I	2	7	2 - 4 (interval 7 - 24)
Paper II	3	39	Several combined
Paper III, IV	1	3	1

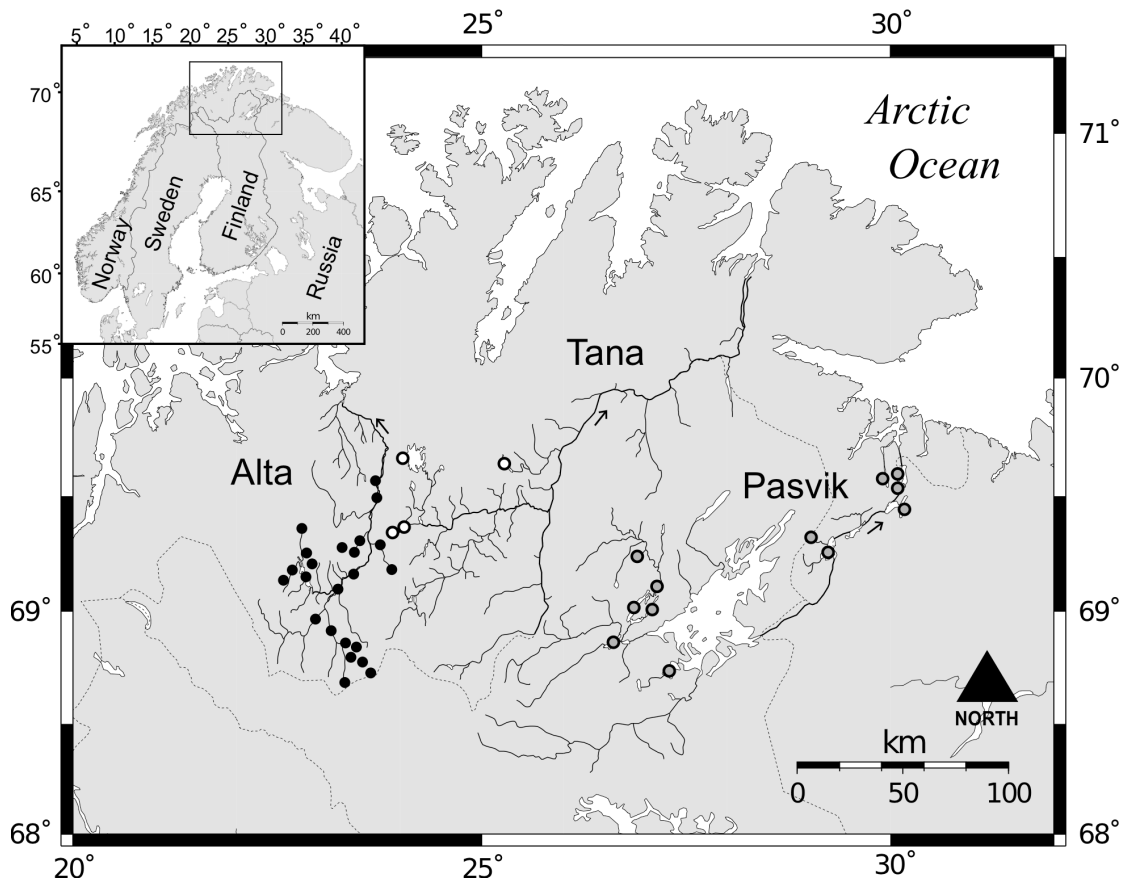


Figure 2. Map of the study area in northern Fennoscandia where whitefish were sampled from the three major watercourses: Alta (black), Tana (white), and Pasvik (grey).

Summary of papers

Paper I

Objectives and questions

To explore the temporal stability of gill raker counts in northern Fennoscandian whitefish populations.

Q1: Is gill raker number a temporally stable morphological trait in northern Fennoscandian whitefish populations?

Q2: Are gill raker number distributions affected by human-induced environmental alterations?

Results and discussion

The temporal stability of the distribution of gill raker number in European whitefish populations was monitored through a long-term study of seven subarctic lakes. Both monomorphic and polymorphic populations of whitefish showed a high consistency in their gill raker number throughout timeframes from seven up to 24 years. Small but significant changes were observed in only three out of 11 populations (morphs within lakes). The absolute differences within these populations were very small (1-2 gill rakers) and likely of negligible biological relevance. Even in two lakes subjected to major human-induced ecological changes during the study period (intensive stock reduction of whitefish, and invasion of a zooplanktivore specialist fish species), gill raker counts remained remarkably stable.

The temporal stability in gill raker counts suggests that the studied populations have reached an optimal phenotype, adapted to physical and biological conditions within each lake. This is also evident from the many examples of correlations between gill raker number and trophic ecology in whitefish morphs (e.g. Nilsson, 1958; Amundsen *et al.*, 2004a; Amundsen *et al.*, 2004b; Kahilainen and Østbye, 2006). Indeed, stabilizing selection on the number of gill rakers within whitefish morphs has been identified in both in Lake whitefish (Bernatchez, 2004) and in European whitefish (Østbye *et al.*, 2005b), and recently also within morphs among lakes in northern Fennoscandia (Præbel *et al.*, *submitted*).

Sympatric whitefish morphs in northern Norway are in general partly reproductively isolated, with small but significant genetic differences (Østbye *et al.*, 2006; Præbel *et al.*, *in press*; Præbel *et al.*, *submitted*; Paper III). In polymorphic lakes the sustained bimodality in gill raker distributions reflects maintained divergent selection regimes, and / or reproductive barriers between

the two morphs, since hybrids of different whitefish morphs are known to exhibit intermediate number of gill rakers (Svårdson, 1952).

The altered selection regimes in the humanly disturbed whitefish populations has led to changes in behavior (diet and habitat use), growth, population structure, and life history (Amundsen, 1988a; Amundsen *et al.*, 2002; Bøhn and Amundsen, 2004). However, these documented ecological changes have not become manifested in correlated changes in gill raker numbers, indicating low plasticity, and / or strong divergent selection still acting on this morphological trait. The sustained bimodal gill raker patterns in the ecologically disturbed populations may also indicate that reproductive barriers between morphs are maintained in spite of altered selection regimes.

Conclusions

Q1: Gill raker number is a temporally stable morphological trait in whitefish populations in northern Fennoscandia, and thus a reliable means of identifying genetically based adaptive divergence.

Q2: Gill raker number distributions were remarkably stable in two lakes experiencing profound human induced changes in selection regimes.

Paper II

Objectives and questions

Objective 1: To explore and describe patterns of phenotypic divergence in terms of number of co-existing morphs and modality of the gill raker number distributions of whitefish from three watercourses in northern Fennoscandia.

Objective 2: To explore effects of ecological opportunity and historical contingency on phenotypic divergence in whitefish populations.

Q1: What are the major patterns and different levels of phenotypic divergence in whitefish in northern Fennoscandia?

Q2: Can contemporary environmental factors related to ecological opportunity and post-glacial colonization history predict the observed phenotypic diversity?

Results and discussion

In this study, the phenotypic diversity of European whitefish populations was quantified by the number of co-existing morphs and the distribution of gill raker numbers in 39 lakes in northern Fennoscandia. The level of diversity was compared within and between three sub-arctic watercourses (Alta, Tana and Pasvik), and explored in relation to ecological opportunity (habitat availability and lake productivity) and colonization history (lake elevation and position). Four patterns of gradually increasing phenotypic diversity were identified. Three of these have previously been described: unimodal, bimodal, and trimodal gill raker distributions, representing monomorphic, dimorphic and trimorphic populations respectively (Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006). In addition, a pattern intermediate to the monomorphic and dimorphic populations was recorded in all three watercourses. The variation in gill raker number distributions between populations was almost continuous, from narrow unimodal, to wide multimodal distribution patterns. This continuum of increasing divergence in a single trait may represent different stages in a common evolutionary process, with gradual evolution of specialization, polymorphism and eventually speciation, i.e. a speciation continuum (Smith and Skúlason, 1996; Hendry, 2009; Nosil *et al.*, 2009).

These populations, expressing different levels of divergence, were used to investigate various factors likely to influence progress between different stages along a continuum of increasing diversity. Ecological opportunity, i.e. higher availability of ecological niches, is expected to facilitate population divergence and increase the number of new types formed in an evolutionary divergence process (Losos and Schluter, 2000; Schluter, 2000; Seehausen, 2006a). Accordingly, the most diverse whitefish populations were found in lakes with highest availability of habitat and diet resources, as inferred from lake size and productivity. However, even when ecological opportunity is present, populations may fail to diversify for a number of reasons (for examples see Losos, 2010).

Lakes were colonized at different points in time and by unique groups of individuals following the deglaciation, and limitations related to time for divergence (Coyne and Orr, 2004) and standing genetic variation (Barrett and Schluter, 2008; Caldera and Bolnick, 2008; Schluter and Conte, 2009) are likely to constrain diversification. In northern Fennoscandia, the ice sheet retreated towards the southwest (Andersen and Borns, 1994; Berglund *et al.*, 1996; Kujansuu *et al.*, 1998), and as expected the easternmost (Pasvik) whitefish populations, which have the highest observed genetic variability (Østbye *et al.*, 2006), also had the highest phenotypic diversity. Phenotypic divergence in whitefish populations in the most western watercourse (Alta) may thus be restricted by a combination of low standing genetic variation and limited time for divergence related to colonization history events.

A cumulative logit model correctly classified the level of polymorphism, to the four identified patterns, in 68 % of the whitefish populations based on simple measures of ecological opportunity (lake area and Secchi depth) and post-glacial colonization history (elevation and watercourse). The predictive power was better for the full model compared to models including only ecological opportunity or colonization history measures, which both correctly classified 53 %. Thus, a combination of ecological opportunity and historical contingency seems to limit the potential for diversification in the studied whitefish populations. Which of these is the most important limiting factor will probably differ between populations. These findings add to the common view that adaptive diversification usually results from exposure to ecological opportunity, but also adds to the knowledge of situations in which divergence do not occur. Less diverse whitefish populations were predicted to occur in small lakes with low productivity, further away from the source population and colonized late during the stepwise postglacial colonization process.

Conclusions

Q1. Three major patterns of phenotypic diversity, representing monomorphic, dimorphic and trimorphic populations of whitefish were observed. In addition, a pattern intermediate to the monomorphic and dimorphic populations was present in several lakes. The different whitefish populations could thus be placed on different stages in a continuum of increasing divergence.

Q2. Mechanisms related to both ecological opportunity and historical contingency seemed to limit the potential for phenotypic diversity in the studied whitefish populations.

Paper III and IV

Objectives and questions

Objective: To examine the early phase of a possible divergence beyond the classical pelagic-littoral dichotomy in European whitefish

Q1: To what extent are profundal resources utilized in lakes with only DR and LSR whitefish morphs, i.e. where the profundal specialist morph (SSR) have not been recorded? (Paper III)

Q2: Are resource specialist groups within the LSR morph morphologically adapted to different environments? (Paper III, IV)

Q3: Are resource specialist groups within the LSR morph genetically differentiated? (Paper III)

Q4: Is divergent natural selection a likely mechanism driving and / or maintaining the intramorph divergence? (Paper III, IV)

Results and discussion

Based on the suggested within-lake origin of sympatric LSR and DR whitefish morphs (Østbye *et al.*, 2006), a similar possible origin of the SSR morph could be sympatric ecological divergence within the LSR morph. To elaborate this idea we implemented ecological “in-depth” studies of the LSR morph in three replicate lakes in search for early signs of a possible divergence to utilize profundal resources. Only lakes where LSR occur sympatrically with DR morphs were included, since this is the situation in all lakes where the SSR morph has been recorded. Based on stomach content data (recent diet) and stable isotope analyses of carbon and nitrogen (long-term resource use), the LSR whitefish morph could be divided into two groups, consisting of individuals specializing on either littoral or profundal benthic resources, in all three lakes. This parallel divergence in feeding behavior was closely correlated to habitat use, with 79-100 % of the individuals correctly classified to resource specialist group based on habitat. The differences in foraging behavior between the littoral and profundal LSR whitefish in these three lakes were about the same magnitude as observed between the LSR and a profundal specialist (SSR) morph in lakes where three sympatric whitefish morphs occur (Kahilainen *et al.*, 2003; Harrod *et al.*, 2010). This suggests that the divergence within the LSR morph in this study is ecologically as profound as between separate morphs in other lakes.

The divergence in resource use within the LSR morph was accompanied by significant morphological differences between profundal and littoral resource specialists, paralleled in three different lakes. Whitefish specializing on profundal resources had fewer gill rakers, a more robust body shape, with relatively large head compared to body size, large eyes dorsally positioned, and long pectoral fins, while littoral specialists had larger number of gill rakers, a more slender body shape, smaller head and eyes, and shorter pectoral fins. The

morphology of the profundal whitefish specialists resembled the phenotypes of profundal specialist morphs of whitefish (SSR) (Kahilainen and Østbye, 2006; Harrod *et al.*, 2010) and other species (Klemetsen *et al.*, 2002; Zimmerman *et al.*, 2006), which further support an adaptive significance of the observed morphological characteristics. Genetic analyses indicated that the two ecological and morphological sub-groups of LSR were partly reproductively isolated in all three lakes. The genetic differences were significant but weak, which may indicate recently evolved reproductive barriers and ongoing gene flow.

In addition, an expected more profound ecological, morphological and genetic divergence between sympatric LSR and DR whitefish morphs (e.g. Østbye *et al.* 2006; Harrod *et al.* 2010) was confirmed. The clear differences between the DR and LSR morphs, and the divergence within the LSR morph between littoral and profundal resources were paralleled in all three lakes included in this study. Such parallels indicate that similar divergent natural selection are driving and / or maintaining the ecological, morphological and genetic divergence between the DR and LSR morphs, and within the LSR whitefish morph (Endler, 1986; Schluter, 2000). Altogether, these results illustrate a possible incipient divergence process towards the evolution of a profundal specialist morph.

Conclusions

Q1: Profundal habitats and food resources were regularly utilized by specialized individuals of the LSR whitefish morph in three different lakes.

Q2: Parallel morphological adaptations to littoral and profundal resource utilization were observed in the three lakes. The morphology of the profundal whitefish specialists resembled that of other profundal specialist fishes, which suggest an adaptive significance of the observed morphological characteristics.

Q3: Significant but weak genetic differences were observed between littoral and profundal resource specialists in all three lakes.

Q4: Replicated divergence in resource utilization, morphology, and genetics within three different lakes suggests that divergent natural selection is an important factor for the observed patterns. Similar selective regimes within lakes may thus be driving and / or maintaining the documented differences both between LSR and DR morphs, and between littoral and profundal specialist groups of LSR whitefish.

Discussion

Parallel adaptive divergence

Whitefish is part of the most extensive example of replicated adaptive divergence, i.e. that of divergence of postglacial fish species into pelagic and littoral morphs (reviewed in Schluter and McPhail, 1993; Robinson and Wilson, 1994; Schluter, 2000; Snorrason and Skúlason, 2004). Whitefish in northern Fennoscandian lakes have repeatedly radiated into specialist morphs for all three principal lake habitats, the pelagic, littoral and profundal zones (Kahilainen *et al.*, 2004; Harrod *et al.*, 2010; Paper II), which strongly suggest predictability of divergence also beyond the common pelagic-littoral dichotomy. Specialism to utilize profundal resources was evident in several lakes (Paper II, III, IV), and the profundal specialists (SSR) always co-occurred with both pelagic (DR) and littoral (LSR) specialists. Within-lake postglacial divergence has been suggested to be the most probable origin of the sympatric DR and LSR morphs in northern Fennoscandian lakes (Østbye *et al.*, 2005a; Østbye *et al.*, 2006). Less is known about the origin of the SSR morph, but recent genetic analyses document reproductive isolation and suggest replicated within-lake divergence from the LSR morph as the most likely origin (Præbel *et al.*, *submitted*). Examples of divergence of fishes into all three principal lake niches are still rare, but has been documented in round whitefish (*Prosopium* sp.) in Alaska (Lindsey, 1981) and in Arctic charr (reviewed in Klemetsen, 2010).

This thesis addresses the first step in an ecological speciation process, namely the importance of ecologically-based natural selection driving adaptive divergence. Correlations between phenotypes and their environment are commonly used to indicate that the observed morphological traits are adaptive (Schluter, 2000). In whitefish, generally the number of gill rakers together with stomach contents and / or habitat utilization has been used to infer phenotype-environment correlations between morphs (e.g. Amundsen, 1988b; Amundsen *et al.*, 2004a; Amundsen *et al.*, 2004b; Kahilainen *et al.*, 2011). This type of correlations was extended to include long-term resource use and body morphology in Paper III and IV. In accordance with the earlier results, differences in short- and long-term resource use (stomach contents and stable isotope ratios, respectively) were related to gill raker number and overall appearance of the DR and LSR morphs (Paper III) (see also Harrod *et al.*, 2010). In addition, differences in foraging behavior and habitat choice were related to gill raker number and body morphology also within the LSR morph, resulting in two ecological sub-groups specializing on littoral and profundal resources, respectively (Paper III, IV). The morphological characteristics of profundal specializing LSR whitefish were similar to the profundal specialist morphs of

whitefish (Kahilainen and Østbye, 2006; Harrod *et al.*, 2010) and other species (Klemetsen *et al.*, 2002; Zimmerman *et al.*, 2006), which further suggest an adaptive value of the observed morphological traits (Paper III, IV). Altogether, results from the present thesis suggest that the observed phenotypic differences are adaptations to the three principal habitats in lakes, the pelagic, littoral and profundal zones.

The observed morphological differences between morphs and between resource specialists within the LSR morph are likely related to both genetic divergence and phenotypic plasticity in response to different environments. The relative contribution of the two sources may vary between traits, and in whitefish gill raker number is considered to have a high genetic component (Svårdson, 1952, 1979; Bernatchez, 2004). Indeed, low plasticity in the number of gill rakers was also revealed in the present whitefish populations based on temporal stability in distributions, even during severe changes in selection regimes (Paper I). Plastic responses may certainly also be adaptive, and in some cases polymorphism may result primarily from phenotypic plasticity (e.g. Hindar and Jonsson, 1993; Mittelbach *et al.*, 1999; Adams and Huntingford, 2004). However, since the whitefish morphs are genetically differentiated (Østbye *et al.*, 2006; Præbel *et al.*, *in press*; Præbel *et al.*, *submitted*; Paper III), plasticity is ruled out as the only explanation for the observed polymorphisms.

Patterns of parallel divergence in ecology, morphology, and genetics in northern Fennoscandian whitefish have now been documented for an increasing number of lakes, both between separate morphs (e.g. Amundsen *et al.*, 2004b; Østbye *et al.*, 2006; Harrod *et al.*, 2010; Paper I, II, III), and between littoral and profundal foraging specialists within the LSR morph (Paper III, IV). Such evolution of similar traits under similar environmental conditions provide strong evidence that natural selection is crucial in generating and / or maintaining the observed differences (Endler, 1986; Schluter, 2000). Strong divergent selection acting on gill raker traits was also suggested based on the stable bimodal gill raker distributions within (Paper I) and between lakes (Paper II). Recently, this was confirmed by between morph P_{ST} (gill raker number) - F_{ST} (microsatellites) comparisons, indicating that divergent natural selection maintained the difference in number of gill rakers between morphs (Præbel *et al.*, *submitted*). Divergent natural selection caused by frequency-dependent competition for resources (e.g. diet and habitat) is a major force in ecological population divergence in general (Schluter, 2000), and has earlier also been suggested as a likely mechanism in the sympatric divergence of the DR and LSR whitefish morphs in northern Fennoscandia (e.g. Amundsen *et al.*, 2004b; Østbye *et al.*, 2006). Resource competition between sympatric DR and LSR morphs has been inferred from restricted resource use of the LSR morph in sympatric compared to allopatric conditions (Amundsen *et al.*, 2004b). In allopatry, individuals of the

LSR morph are intermediate in body shape between sympatric DR and LSR morphs and exploit both pelagic and benthic resources (Amundsen *et al.*, 2004b; Harrod *et al.*, 2010). Resource competition is likely to be important also for the divergence between the LSR and SSR morphs. In the absence of the SSR morph, the LSR whitefish are able to utilize a combination of littoral and profundal resources (Paper III). In sympatry in contrast, the LSR whitefish is a littoral specialist restricted from extensive use of profundal habitats and prey resources (Harrod *et al.*, 2010), likely as a result of competitive interactions with the SSR whitefish morph.

Predicting the level of diversity

Whitefish in northern Fennoscandian lakes have repeatedly radiated into specialist morphs for all three principal lake habitats, the pelagic, littoral and profundal zones (Kahilainen *et al.*, 2004; Harrod *et al.*, 2010; Paper II). In addition, intermediate stages were identified both between mono- and dimorphic, and between di- and trimorphic populations (Paper II, III, IV). Imagining that the adaptive divergence process occur in a similar way in all lakes, this diversity of populations could represent “snapshots” at different points of time in a common evolutionary process. Thus, these populations may constitute different stages in a continuum of increasing diversity, and the differences between them may elucidate important factors promoting or constraining divergence and ecological speciation (e.g. Paper II). However, populations are not necessarily in a process of increasing divergence and speciation, and each case could also represent a stable situation where special requirements are needed for transition to the next stage that are not present in the current situation (Hendry *et al.*, 2009; Bolnick, 2011).

The results in this thesis suggest several ecological and non-ecological factors that may be important for progress along a continuum of diversity in the present whitefish populations. These factors may also help to explain differences in whitefish diversity between northern Fennoscandian lakes. In species poor postglacial lakes, there is increasing evidence that ecological opportunity in combination with high intraspecific competition promotes adaptive divergence of fish (Skúlason and Smith, 1995; Lu and Bernatchez, 1999; Robinson *et al.*, 2000; Vamosi, 2003; Bolnick, 2004). Whitefish is able to utilize a wide range of lake resources and tend to diversify into specialists of the available major lake environments (i.e. pelagic, littoral, profundal) in each lake (Paper II, III, IV). Many abiotic and biotic factors differ between these principal environments, e.g. light condition, temperature, physical complexity, availability and type of prey, and predation risk. Thus when the opportunity is there, multifarious selection, related to a number of different niche axes, is expected to promote adaptive

divergence to these principal lake environments (Nosil and Rundle, 2009; Nosil, 2012). Phenotypic diversity in northern Fennoscandian whitefish populations was successfully predicted by ecological opportunity as described by lake size and productivity (Paper II). Further, divergence in these whitefish populations is likely the result of intraspecific resource competition (Amundsen *et al.*, 2004b), and apparently not constrained by the co-occurrence of several other fish species (Paper II). Possible diversifying effects of other ecological interactions, e.g. predation (Nosil and Crespi, 2006; Langerhans *et al.*, 2007; Meyer and Kassen, 2007), are interesting subjects for future studies.

Higher phenotypic diversity of whitefish populations could also be explained by non-ecological factors such as longer time for divergence, higher genetic diversity, and larger lake size allowing for larger distances and lower gene flow between diverging morphs (Paper II). In addition, temporal stability of abiotic and biotic environmental factors, including persistent intraspecific competition, is expected to facilitate adaptive divergence and reduce the capability of plastic responses (Smith, 1993; Snorrason and Skúlason, 2004; Svanbäck *et al.*, 2009).

Stages along a speciation continuum

A distinct and predictable order of different stages in evolutionary diversification has been suggested for several species (reviewed in Streebman and Danley, 2003; Ackerly *et al.*, 2006). Closely related taxa are likely to radiate in more similar ways since they share similar developmental and genetic systems. Thus, the framework presented for the evolution of different morphs of another salmonid, the Arctic charr (*Salvelinus alpinus*) (Skúlason *et al.*, 1999; Snorrason and Skúlason, 2004), may be representative also for whitefish adaptive divergence. Both Arctic charr and whitefish are described as highly plastic and phenotypically variable species (Svårdson, 1957, 1979; Jonsson and Jonsson, 2001; Klemetsen, 2010). A basic assumption in the Arctic charr framework is that lake environments have gone from being instable shortly after the deglaciation, to being more predictable regarding food and habitat resources for fish. Thus, plasticity is expected to be important in the first stages of divergence, similar to more general evolutionary models described by West-Eberhard (1989; 2003).

In the first stage of the Arctic charr model, newly formed lakes were colonized by fish rapidly exploiting a wide range of resources through their behavioral flexibility and plasticity in life history. In the second stage, rapid and extensive diversification of phenotypes occurs in response to behavioral specializations. These discrete resource morphs may at first be the result solely of plastic responses to different environments. In the third stage, polymorphisms may

become genetically assimilated (Waddington, 1953) when environments become more stable and predictable. In the last stage in the model, reproductive isolation between morphs may evolve, e.g. by temporal or spatial segregation in spawning, or selection on mating behavior. (Skúlason *et al.*, 1999; Snorrason and Skúlason, 2004)

These four stages suggested for adaptive radiation in Arctic charr are similar to the idea of four states along the speciation continuum suggested by Hendry (2009). In Figure 3, these two views are combined and exemplified by whitefish systems in northern Fennoscandia. Monomorphic populations of LSR whitefish represent the starting point, stage 1, with continuous variation in morphology and resource use (Amundsen *et al.*, 2004b; Harrod *et al.*, 2010; Paper II). Although not known, genetic differentiation is thought to be absent. In the studies of divergence within sympatric LSR morphs to littoral and profundal specialists, pronounced ecological divergence (diet and habitat use) was related to less distinct differences in morphology, and weak reproductive isolation (Paper III, IV). This may represent stage 2, where divergence in resource use has not become manifested in very distinct morphological adaptations and strong reproductive isolation, thus emphasizing the importance of behavioral flexibility in early stages of divergence. Another, less studied example of stage 2 would be the intermediate monomorphic whitefish populations. They appeared to have gill raker numbers intermediate to the DR and LSR morphs, and also increased morphological variation (Paper II). However, less is known about the ecology and genetics in these populations. Stage 3 could be exemplified by any combination of sympatric whitefish morphs. Between morphs, pronounced adaptive divergence in ecology and morphology are correlated with low levels of genetic differentiation (Østbye *et al.*, 2006; Harrod *et al.*, 2010; Præbel *et al.*, *submitted*; Paper II, III), indicating some, but not irreversible reproductive isolation. To my knowledge, complete and irreversible reproductive isolation (stage 4), such as genetic incompatibilities, has not been documented in northern European whitefish morphs.

Apparently two different stages along the speciation continuum are present in some dimorphic whitefish systems, stage 3 between DR and LSR morphs, and stage 2 between littoral and profundal LSR specialists. Thus, whitefish in the lakes with apparently only two morphologically distinct morphs may be in the process of evolution towards three specialist morphs. However, the evolutionary outcome is not guaranteed to be discrete polymorphism or speciation, as in some systems flexibility and plasticity may rather continue to be favored (Snorrason and Skúlason, 2004). The documented intermediate patterns (stage 2) could be the result of either divergent natural selection (e.g. Rueffler *et al.*, 2006; Bolnick, 2011), or a reversal of the speciation process with a collapse of sympatric morphs (e.g. as observed in Taylor *et al.*, 2006; Seehausen *et al.*, 2008a).

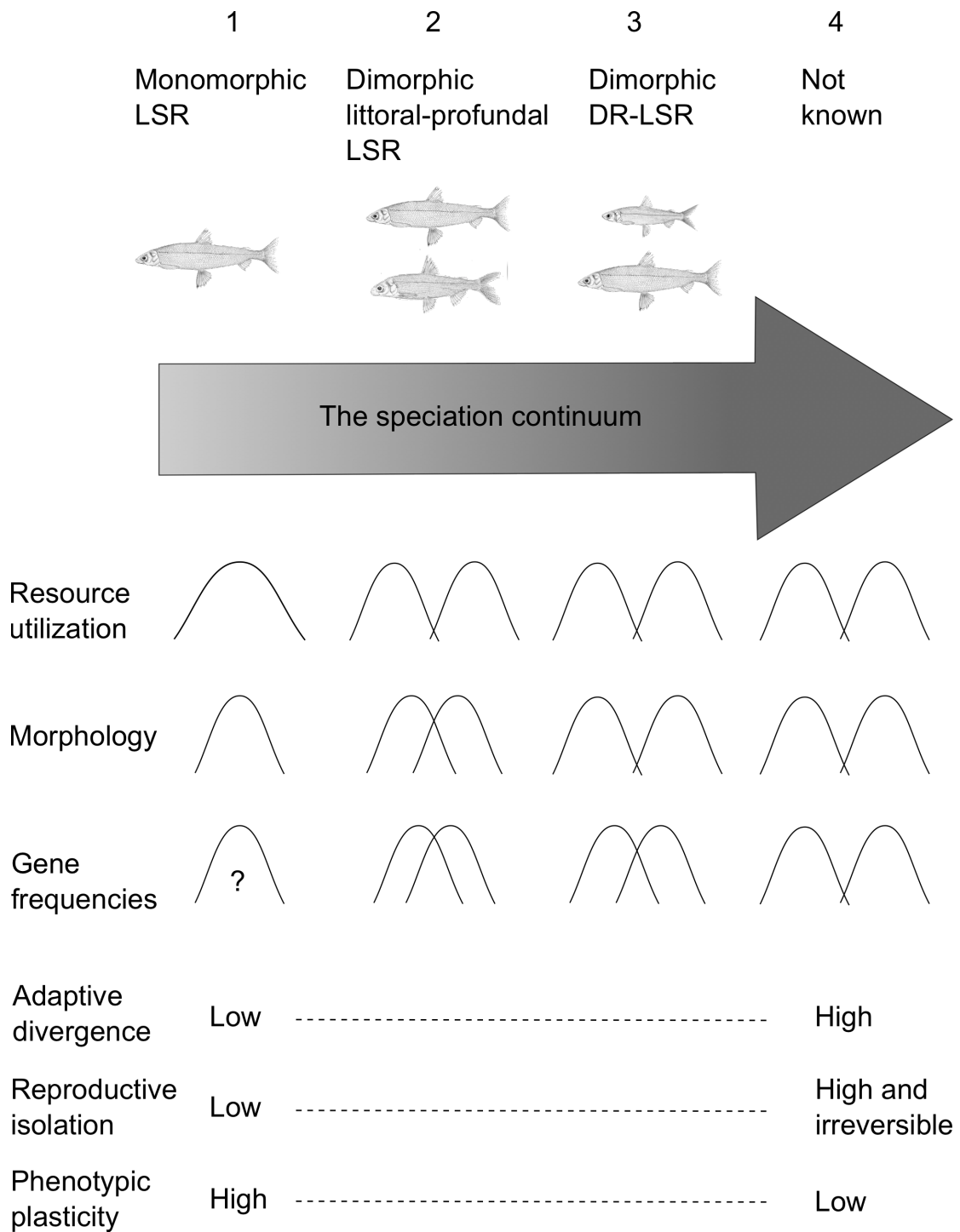


Figure 3. Schematic illustration of the increasingly discrete variation along a speciation continuum in northern European whitefish. Four different stages are described by divergence in resource use, morphology, and gene frequencies. Examples from whitefish exist for the first three stages, and illustrations of divergence for those were based on results from Paper II, III, and IV in addition to Amundsen *et al.* (2004b). Further, theoretical expectations of variation in adaptive divergence, reproductive isolation and importance of phenotypic plasticity are indicated (e.g. Snorrason and Skúlason, 2004; Hendry, 2009; Nosil, 2012).

A reversal of the speciation process has been documented in European whitefish (Vonlanthen *et al.*, 2012) as well as in other fish species (Seehausen, 2006b; Taylor *et al.*, 2006; Seehausen *et al.*, 2008a; Behm *et al.*, 2010). Young species and morph pairs with incomplete reproductive isolation may be especially prone to hybridization following changes in the selection regime (Coyne and Orr, 2004), and human-induced alterations in environmental conditions have likely on several occasions been responsible for the collapse (reviewed in Seehausen *et al.*, 2008a). The ecological collapse between two whitefish morphs, following the introduction of vendace in the Pasvik watercourse, did not seem to increase hybridization between morphs based on gill raker counts (Paper I). However, an increased incidence of genetically identified hybrids between DR and LSR whitefish morphs were recently documented following the vendace invasion in one of the lakes in the watercourse (Bhat *et al.*, 2011), suggesting that the reproductive barriers between the morphs have been degraded.

Whitefish – a case of ecological speciation?

The first step in an ecological speciation process, divergent natural selection driving adaptive divergence, seems to be present in northern Fennoscandian whitefish. For completion of an ecological speciation event, reproductive isolation must subsequently evolve as a consequence of the adaptive divergence to different niches (Schluter, 2001; Rundle and Nosil, 2005). Thus, one prediction of ecological speciation is that gene flow between populations will decrease as adaptive divergence increases (Funk *et al.*, 2006; Nosil and Rundle, 2009). The numerous replicate cases of reproductively isolated whitefish morphs adapted to different environments (e.g. Østbye *et al.*, 2006; Præbel *et al.*, *submitted*) support that adaptive divergence is causing reduced gene flow between morphs (Räsänen and Hendry, 2008). Thus, northern Fennoscandian whitefish constitute a putative example of ecological speciation.

Although the identification of reproductive barriers between whitefish morphs was not the subject of the present thesis, an evaluation of possible isolation mechanisms is useful when considering the diversification of northern Fennoscandian whitefish as a potential example of incipient ecological speciation. The reproductive isolation among sympatric whitefish morphs in northern Fennoscandia has been found to be surprisingly weak (Østbye *et al.*, 2006; Præbel *et al.*, *in press*; Paper III). This is also reflected in high frequencies (approx. 25 – 50 %) of naturally occurring hybrids between DR and LSR whitefish morphs in several lakes (Kim Præbel, pers. com.). In addition, the invasion of vendace in the Pasvik watercourse has apparently led to increased hybridization between DR and LSR whitefish morphs (Bhat *et al.*, 2011), although the gill raker counts so far have remained stable (Paper I). These recent

genetic analyses illustrate the fragility of the reproductive barriers among whitefish morphs and indicate that intrinsic postzygotic isolation (i.e. genetic incompatibilities in hybrids) is unlikely in northern Fennoscandian whitefish. A similar experimental result has been obtained from other European whitefish populations (Woods *et al.*, 2009).

Selection against intermediate hybrids is likely to be an important reproductive barrier, at least in early stages of ecological speciation (e.g. Hatfield and Schluter, 1999; Rundle, 2002). This expectation was supported by the low frequency of individuals with intermediate gill raker numbers in sympatric whitefish morphs (Paper II), and the stability of bimodal gill raker distributions (Paper I). Strong divergent selection on gill raker number (Østbye *et al.*, 2005b; Præbel *et al.*, *submitted*; Paper I, II), or other correlated traits, may thus be important in the evolution of reproductive isolation between whitefish morphs. However, extrinsic postmating isolation is suggested to be insufficient for separate species to evolve since, without any kind of assortative mating, hybrids continue to be produced.

Divergence in location and / or time for spawning is common between morphs of postglacial fish (e.g. Svårdson, 1979; Jonsson and Jonsson, 2001; Østbye *et al.*, 2005b; Vonlanthen *et al.*, 2009; Klemetsen, 2010). Unfortunately, knowledge about time and place of spawning is poor in the present systems, partly related to the difficult weather and ice conditions in the area at the typical spawning time in October and November. Based on catch data from different habitats, sympatric whitefish morphs generally tend to reside in the environment they are best adapted to, i.e. few DR whitefish are caught in benthic habitats and few LSR in the pelagic (e.g. Amundsen *et al.*, 2004b; Harrod *et al.*, 2010; Paper III). Differences between environments in abiotic and biotic factors (e.g. temperature or seasonality of food resources) that affect the timing of maturation may lead to temporal isolation in spawning (Hendry and Day, 2005). Also, spawning time is heritable in several salmonid populations (Hendry and Day, 2005), and is likely to be a magic trait (Servedio *et al.*, 2011) responsible for the evolution of polymorphism in postglacial fish through its indirect effects on developmental rates and morphology (McPhee *et al.*, 2012).

Closing remarks

In this thesis I have documented a continuum of increasing phenotypic diversity, including a gradual divergence to specializations for profundal resources in northern European whitefish populations. The profound ecological and morphological divergence between sympatric morphs is accompanied by relatively weak reproductive isolation. This suggests that selection must be

strong for specialization to divergent environments, since morphs persist in the face of gene flow. The very same ecological conditions driving ecological and morphological divergence in whitefish may also lead to reproductive isolation, and thus ecologically based speciation.

Speciation is undoubtedly a complex process, which can be described by different attributes related to the geographic context (i.e. allopatric vs. sympatric) or diversifying mechanisms (e.g. ecological vs. nonecological, adaptive vs. nonadaptive, natural vs. sexual selection, selection vs. drift). Moreover, many different geographical contexts and mechanisms may be involved in any individual speciation event. Recent opinions favor a more continuous view of speciation as a process occurring in time and space (Dieckmann *et al.*, 2004; Fitzpatrick *et al.*, 2009; Smadja and Butlin, 2011; Butlin *et al.*, 2012), and the concept of a “speciation continuum”, where the different whitefish populations may represent different points of time in a common evolutionary process, is certainly also a simplification. In addition, speciation is by no means a “one-way street”, and populations could also represent stable situations at intermediate stages along the speciation continuum. Northern Fennoscandian whitefish populations represent a promising study system of incipient ecological speciation, potentially also including examples of speciation reversal. Hopefully these fish will continue to add to our knowledge about “that mystery of mysteries” (Darwin, 1859) and the astonishing diversity of life!

Conclusions

1. Gill raker number is a temporally stable morphological trait in whitefish populations in northern Fennoscandia, even during major human-induced ecological changes. Thus, this trait may be used for identification of genetically based adaptive divergence. (Paper I)
2. An almost continuous variation of increasing phenotypic diversity was documented among a large number of whitefish populations. Based on gill raker number distributions, three major patterns of diversity, representing monomorphic, dimorphic and trimorphic populations of whitefish, were observed. In addition, a pattern intermediate to the monomorphic and dimorphic populations was present in several lakes. (Paper II)
3. Mechanisms related to both ecological opportunity and historical contingency seemed to limit the potential for phenotypic divergence in the studied whitefish populations. Less diversity was documented in small lakes with low productivity, further away from the source population, where populations were founded late during the stepwise postglacial colonization process. (Paper II)
4. Profundal resources were regularly used by morphologically adapted and partly reproductively isolated groups of LSR whitefish within three different lakes. This parallel divergence could indicate an incipient evolution towards a profundal specialist morph in some dimorphic whitefish populations. (Paper III, IV)

References

- Ackerly, D.D., Schwilk, D.W. and Webb, C.O. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. *Ecology*, **87**: S50-S61.
- Adams, C.E. and Huntingford, F.A. 2004. Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biol J Linn Soc*, **81**: 611-618.
- Amundsen, P.-A. 1988a. Effects of an intensive fishing programme on age structure, growth and parasite infection of stunted whitefish (*Coregonus lavaretus* L. s.l.) in Lake Stuurajavri, northern Norway. *Finn Fish Res*, **9**: 425-434.
- Amundsen, P.-A. 1988b. Habitat and food segregation of two sympatric populations of whitefish (*Coregonus lavaretus* L. s.l.) in Stuurajavri, northern Norway. *Nord J Freshwater Res*, **64**: 67-73.
- Amundsen, P.-A., Bøhn, T. and Våga, G.H. 2004a. Gill raker morphology and feeding ecology of two sympatric morphs of European whitefish (*Coregonus lavaretus*). *Ann Zool Fenn*, **41**: 291-300.
- Amundsen, P.-A., Knudsen, R., Klemetsen, A. and Kristoffersen, R. 2004b. Resource competition and interactive segregation between sympatric whitefish morphs. *Ann Zool Fenn*, **41**: 301-307.
- Amundsen, P.-A., Kristoffersen, R., Knudsen, R. and Klemetsen, A. 2002. Long-term effects of a stock depletion programme: the rise and fall of a rehabilitated whitefish population. *Advanc Limnol*, **57**: 577-588.
- Andersen, B.G. and Borns, H.W. 1994. *The ice age world*. Oslo: Scandinavian University Press.
- Barrett, R.D.H. and Schluter, D. 2008. Adaptation from standing genetic variation. *Trends Ecol Evol*, **23**: 38-44.
- Behm, J.E., Ives, A.R. and Boughman, J.W. 2010. Breakdown in postmating isolation and the collapse of a species pair through hybridization. *Am Nat*, **175**: 11-26.
- Berglund, B.E., Birks, H.J.B., Ralska-Jasiewiczowa, M. and Wright, H.E. 1996. *Palaeoecological events during the last 15 000 years*. Chichester: John Wiley & Sons Ltd.
- Bernatchez, L. 2004. Ecological theory of adaptive radiation. An empirical assessment from coregonine fishes (Salmoniformes). In *Evolution illuminated, salmon and their relatives* (A.P. Hendry and S.C. Stearns, eds), pp. 175-207. New York, NY: Oxford University Press.
- Bernatchez, L., Renaut, S., Whiteley, A.R., Derome, N., Jeukens, J., Landry, L., Lu, G., Nolte, A.W., Østbye, K., Rogers, S.M. and St-Cyr, J. 2010. On the origin of species: insights from the ecological genomics of lake whitefish. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**: 1783-1800.
- Berner, D., Grandchamp, A.C. and Hendry, A.P. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. *Evolution*, **63**: 1740-1753.

- Berner, D., Roesti, M., Hendry, A.P. and Salzburger, W. 2010. Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Mol Ecol*, **19**: 4963-4978.
- Bhat, S., Amundsen, P.-A., Knudsen, R., Bernatchez, L., Gjelland, K.Ø., Fevolden, S.-E. and Præbel, K. 2011. Is breakdown of reproductive isolation between a species-pair of European whitefish caused by a biological invasion? *Oral presentation at the 11th international symposium on the biology and management of coregonid fishes, Mondsee, Austria*: 29-30 Sept. 2011.
- Bøhn, T. and Amundsen, P.-A. 2004. Invasion-mediated changes in the population biology of a dimorphic whitefish *Coregonus lavaretus* population. *Ann Zool Fenn*, **41**: 125-136.
- Bolnick, D.I. 2004. Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution*, **58**: 608-618.
- Bolnick, D.I. 2011. Sympatric speciation in threespine stickleback: why not? *International Journal of Ecology*, **2011**: 1-15.
- Bolnick, D.I. and Fitzpatrick, B.M. 2007. Sympatric speciation: models and empirical evidence. *Annu Rev Ecol Evol Syst*, **38**: 459-487.
- Butlin, R., Debelle, A., Kerth, C., Snook, R.R., Beukeboom, L.W., Cajas, R.F.C., Diao, W., Maan, M.E., Paolucci, S., Weissing, F.J., van de Zande, L., Hoikkala, A., Geuverink, E., Jennings, J., Kankare, M., Knott, K.E., Tyukmaeva, V.I., Zoumadakis, C., Ritchie, M.G., Barker, D., Immonen, E., Kirkpatrick, M., Noor, M., Garcia, C.M., Schmitt, T. and Schilthuizen, M. 2012. What do we need to know about speciation? *Trends Ecol Evol*, **27**: 27-39.
- Caldera, E.J. and Bolnick, D.I. 2008. Effects of colonization history and landscape structure on genetic variation within and among threespine stickleback (*Gasterosteus aculeatus*) populations in a single watershed. *Evol Ecol Res*, **10**: 575-598.
- Coyne, J.A. and Orr, H.A. 1989. Patterns of speciation in *Drosophila*. *Evolution*, **43**: 362-381.
- Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA.: Sinauer Associates.
- Crispo, E., Bentzen, P., Reznick, D.N., Kinnison, M.T. and Hendry, A.P. 2006. The relative influence of natural selection and geography on gene flow in guppies. *Mol Ecol*, **15**: 49-62.
- Darwin, C. 1859. *The origin of species*. London: J. Murray.
- Day, T., Pritchard, J. and Schluter, D. 1994. A comparison of two sticklebacks. *Evolution*, **48**: 1723-1734.
- Dieckmann, U., Doebeli, M., Metz, J.A.J. and Tautz, D. (eds) 2004. *Adaptive speciation*. Cambridge, UK: Cambridge University Press.
- Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F. and Jetz, W. 2008. Homage to Linnaeus: how many parasites? how many hosts? *PNAS*, **105**: 11482-11489.
- Endler, J.A. 1986. *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
- Fitzpatrick, B.M. 2012. Underappreciated consequences of phenotypic plasticity for ecological speciation. *International Journal of Ecology*, **2012**: 1-12.
- Fitzpatrick, B.M., Fordyce, J.A. and Gavrilets, S. 2009. Pattern, process and geographic modes of speciation. *J Evol Biol*, **22**: 2342-2347.

- Foote, C.J., Moore, K., Stenberg, K., Craig, K.J., Wenburg, J.K. and Wood, C.C. 1999. Genetic differentiation in gill raker number and length in sympatric anadromous and nonanadromous morphs of sockeye salmon, *Oncorhynchus nerka*. *Environ Biol Fishes*, **54**: 263-274.
- Funk, D.J., Nosil, P. and Etges, W.J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *PNAS*, **103**: 3209-3213.
- Funk, W.C., Tyburczy, J.A., Knudsen, K.L., Lindner, K.R. and Allendorf, F.W. 2005. Genetic basis of variation in morphological and life-history traits of a wild population of pink salmon. *J Hered*, **96**: 24-31.
- Gavrilets, S. and Losos, J.B. 2009. Adaptive radiation: contrasting theory with data. *Science*, **323**: 732-737.
- Grant, P.R. and Grant, B.R. 2008. *How and why species multiply: the radiation of Darwin's finches*. Princeton, NJ.: Princeton University Press.
- Harrod, C., Mallela, J. and Kahilainen, K.K. 2010. Phenotype-environment correlations in a putative whitefish adaptive radiation. *J Anim Ecol*, **79**: 1057-1068.
- Hatfield, T. 1997. Genetic divergence in adaptive characters between sympatric species of stickleback. *Am Nat*, **149**: 1009-1029.
- Hatfield, T. and Schluter, D. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution*, **53**: 866-873.
- Hendry, A.P. 2009. Ecological speciation! Or the lack thereof? *Can J Fish Aquat Sci*, **66**: 1383-1398.
- Hendry, A.P., Bolnick, D.I., Berner, D. and Peichel, C.L. 2009. Along the speciation continuum in sticklebacks. *J Fish Biol*, **75**: 2000-2036.
- Hendry, A.P. and Day, T. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. *Mol Ecol*, **14**: 901-916.
- Himberg, K.J.M. and Lehtonen, H. 1995. Systematics and nomenclature of coregonid fishes, particularly in Northwest Europe. *Advanc Limnol*, **46**: 39-47.
- Hindar, K. and Jonsson, B. 1993. Ecological polymorphism in Arctic charr. *Biol J Linn Soc*, **48**: 63-74.
- Hudson, A.G., Vonlanthen, P. and Seehausen, O. 2011. Rapid parallel adaptive radiations from a single hybridogenic ancestral population. *Proc R Soc B*, **278**: 58-66.
- Jonsson, B. and Jonsson, N. 2001. Polymorphism and speciation in Arctic charr. *J Fish Biol*, **58**: 605-638.
- Kaeuffer, R., Peichel, C.L., Bolnick, D.I. and Hendry, A.P. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution*, **66**: 402-418.
- Kahilainen, K., Lehtonen, H. and Könönen, K. 2003. Consequence of habitat segregation to growth rate of two sparsely rakered whitefish (*Coregonus lavaretus* (L.)) forms in a subarctic lake. *Ecol Freshw Fish*, **12**: 275-285.
- Kahilainen, K., Malinen, T., Tuomaala, A. and Lehtonen, H. 2004. Diel and seasonal habitat and food segregation of three sympatric *Coregonus lavaretus* forms in a subarctic lake. *J Fish Biol*, **64**: 418-434.

- Kahilainen, K. and Østbye, K. 2006. Morphological differentiation and resource polymorphism in three sympatric whitefish *Coregonus lavaretus* (L.) forms in a subarctic lake. *J Fish Biol*, **68**: 63-79.
- Kahilainen, K.K., Siwertsson, A., Gjelland, K.Ø., Knudsen, R., Bøhn, T. and Amundsen, P.-A. 2011. The role of gill raker number variability in adaptive radiation of coregonid fish. *Evol Ecol*, **25**: 573-588.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill, C.E., Hoang, A., Gibert, P. and Beerli, P. 2001. The strength of phenotypic selection in natural populations. *Am Nat*, **157**: 245-261.
- Klemetsen, A. 2010. The charr problem revisited: exceptional phenotypic plasticity promotes ecological speciation in postglacial lakes. *Freshw Rev*, **3**: 49-74.
- Klemetsen, A., Elliott, J.M., Knudsen, R. and Sørensen, P. 2002. Evidence for genetic differences in the offspring of two sympatric morphs of Arctic charr. *J Fish Biol*, **60**: 933-950.
- Kujansuu, R., Eriksen, B. and Grönlund, T. 1998. Lake Inarijärvi, northern Finland: sedimentation and late Quaternary evolution. *Geological Survey of Finland, Report of Investigation*, **143**: 1-22.
- Langerhans, R.B., Gifford, M.E. and Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. *Evolution*, **61**: 2056-2074.
- Lindsey, C.C. 1981. Stocks are chameleons: plasticity in gill-rakers of coregonid fishes. *Can J Fish Aquat Sci*, **38**: 1497-1506.
- Losos, J.B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *Am Nat*, **175**: 623-639.
- Losos, J.B. and Schluter, D. 2000. Analysis of an evolutionary species-area relationship. *Nature*, **408**: 847-850.
- Lu, G.Q. and Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*, **53**: 1491-1505.
- Mallet, J., Beltran, M., Neukirchen, W. and Linares, M. 2007. Natural hybridization in heliconiine butterflies: the species boundary as a continuum. *BMC Evol Biol*, **7**.
- May, R.M. 1994. Conceptual aspects of the quantification of the extent of biological diversity. *Philos T Roy Soc B*, **345**: 13-20.
- May, R.M. 2011. Why worry about how many species and their loss? *PLoS Biol*, **9**.
- McPhee, M.V., Noakes, D.L.G. and Allendorf, F.W. 2012. Developmental rate: a unifying mechanism for sympatric divergence in postglacial fishes? *Curr Zool*, **58**: 21-34.
- Merrill, R.M., Gompert, Z., Dembeck, L.M., Kronforst, M.R., McMillan, W.O. and Jiggins, C.D. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. *Evolution*, **65**: 1489-1500.
- Meyer, J.R. and Kassen, R. 2007. The effects of competition and predation on diversification in a model adaptive radiation. *Nature*, **446**: 432-435.
- Mittelbach, G.C., Osenberg, C.W. and Wainwright, P.C. 1999. Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution? *Evol Ecol Res*, **1**: 111-128.
- Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B. and Worm, B. 2011. How many species are there on earth and in the ocean? *PLoS Biol*, **9**.

- Nilsson, N.-A. 1958. On the food competition between two species of *Coregonus* in a north-Swedish lake. *Rep Inst Freshw Res Drott*, **39**: 146-161.
- Nosil, P. 2012. *Ecological speciation*. Oxford: Oxford University Press.
- Nosil, P. and Crespi, B.J. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *PNAS*, **103**: 9090-9095.
- Nosil, P., Crespi, B.J. and Sandoval, C.P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, **417**: 440-443.
- Nosil, P., Harmon, L.J. and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol Evol*, **24**: 145-156.
- Nosil, P. and Rundle, H.D. 2009. Ecological speciation: natural selection and the formation of new species. In *The princeton guide to ecology* (S.A. Levin, ed), pp. 134-142. Oxford, UK: Princeton University Press.
- Nosil, P. and Schluter, D. 2011. The genes underlying the process of speciation. *Trends Ecol Evol*, **26**: 160-167.
- Østbye, K., Amundsen, P.-A., Bernatchez, L., Klemetsen, A., Knudsen, R., Kristoffersen, R., Næsje, T.F. and Hindar, K. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol Ecol*, **15**: 3983-4001.
- Østbye, K., Bernatchez, L., Næsje, T.F., Himberg, K.J.M. and Hindar, K. 2005a. Evolutionary history of the European whitefish *Coregonus lavaretus* (L.) species complex as inferred from mtDNA phylogeography and gill-raker numbers. *Mol Ecol*, **14**: 4371-4387.
- Østbye, K., Næsje, T.F., Bernatchez, L., Sandlund, O.T. and Hindar, K. 2005b. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. *J Evol Biol*, **18**: 683-702.
- Peccoud, J., Ollivier, A., Plantegenest, M. and Simon, J.C. 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. *PNAS*, **106**: 7495-7500.
- Pfennig, D.W., Wund, M.A., Snell-Rood, E.C., Cruickshank, T., Schlichting, C.D. and Moczek, A.P. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol Evol*, **25**: 459-467.
- Pigliucci, M., Murren, C.J. and Schlichting, C.D. 2006. Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol*, **209**: 2362-2367.
- Præbel, K., Knudsen, R., Siwertsson, A., Kahilainen, K.K., Østbye, K., Peruzzi, S., Fevolden, S.-E. and Amundsen, P.-A. *submitted*. Ecological speciation in postglacial European whitefish: rapid adaptive radiations into the littoral, pelagic and profundal lake habitats. *Mol Ecol*.
- Præbel, K., Westgaard, J.I., Amundsen, P.-A., Siwertsson, A., Knudsen, R., Kahilainen, K.K. and Fevolden, S.-E. *in press*. A diagnostic tool for efficient analysis of population structure, hybridization and conservation status of European whitefish (*Coregonus lavaretus* (L.)) and vendace (*C. albula* (L.)). *Fundam Appl Limnol - Advanc Limnol*.
- Price, T.D., Qvarnström, A. and Irwin, D.E. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proc R Soc Lond B*, **270**: 1433-1440.
- Räsänen, K. and Hendry, A.P. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecol Lett*, **11**: 624-636.

- Robinson, B.W. and Wilson, D.S. 1994. Character release and displacement in fishes - a neglected literature. *Am Nat*, **144**: 596-627.
- Robinson, B.W., Wilson, D.S. and Margosian, A.S. 2000. A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). *Ecology*, **81**: 2799-2812.
- Rosenblum, E.B. and Harmon, L.J. 2011. "Same same but different": replicated ecological speciation at White Sands. *Evolution*, **65**: 946-960.
- Rueffler, C., Van Dooren, T.J.M., Leimar, O. and Abrams, P.A. 2006. Disruptive selection and then what? *Trends Ecol Evol*, **21**: 238-245.
- Rundle, H.D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. *Evolution*, **56**: 322-329.
- Rundle, H.D., Nagel, L., Boughman, J.W. and Schluter, D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science*, **287**: 306-308.
- Rundle, H.D. and Nosil, P. 2005. Ecological speciation. *Ecol Lett*, **8**: 336-352.
- Sanderson, S.L., Cheer, A.Y., Goodrich, J.S., Graziano, J.D. and Callan, W.T. 2001. Crossflow filtration in suspension-feeding fishes. *Nature*, **412**: 439-441.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Philos T Roy Soc B*, **351**: 807-814.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol Evol*, **16**: 372-380.
- Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science*, **323**: 737-741.
- Schluter, D. and Conte, G.L. 2009. Genetics and ecological speciation. *PNAS*, **106**: 9955-9962.
- Schluter, D. and McPhail, J.D. 1993. Character displacement and replicate adaptive radiation. *Trends Ecol Evol*, **8**: 197-200.
- Seehausen, O. 2004. Hybridization and adaptive radiation. *Trends Ecol Evol*, **19**: 198-207.
- Seehausen, O. 2006a. African cichlid fish: a model system in adaptive radiation research. *Proc R Soc B*, **273**: 1987-1998.
- Seehausen, O. 2006b. Conservation: losing biodiversity by reverse speciation. *Curr Biol*, **16**: R334-R337.
- Seehausen, O. 2009. Progressive levels of trait divergence along a 'speciation transect' in Lake Victoria cichlid fish *Pundamilia*. In *Speciation and patterns of diversity* (R. Butlin, J. Bridle and D. Schluter, eds), pp. 155-176. Cambridge: Cambridge University Press.
- Seehausen, O., Takimoto, G., Roy, D. and Jokela, J. 2008a. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol Ecol*, **17**: 30-44.
- Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R., van der Sluijs, I., Schneider, M.V., Maan, M.E., Tachida, H., Imai, H. and Okada, N. 2008b. Speciation through sensory drive in cichlid fish. *Nature*, **455**: 620-627.
- Servedio, M.R. and Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Annu Rev Ecol Evol Syst*, **34**: 339-364.
- Servedio, M.R., Van Doorn, G.S., Kopp, M., Frame, A.M. and Nosil, P. 2011. Magic traits in speciation: 'magic' but not rare? *Trends Ecol Evol*, **26**: 389-397.

- Skúlason, S. and Smith, T.B. 1995. Resource polymorphisms in vertebrates. *Trends Ecol Evol*, **10**: 366-370.
- Skúlason, S., Snorrason, S.S. and Jonsson, B. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on arctic charr. In *Evolution of biological diversity* (A.E. Magurran and R.M. May, eds), pp. 70-92. Oxford, UK: Oxford University Press.
- Smadja, C.M. and Butlin, R.K. 2011. A framework for comparing processes of speciation in the presence of gene flow. *Mol Ecol*, **20**: 5123-5140.
- Smith, T.B. 1993. Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*. *Nature*, **363**: 618-620.
- Smith, T.B. and Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu Rev Ecol Syst*, **27**: 111-133.
- Snorrason, S.S. and Skúlason, S. 2004. Adaptive speciation in northern freshwater fishes. In *Adaptive speciation* (U. Dieckmann, M. Doebeli, J.A.J. Metz and D. Tautz, eds), pp. 210-228. Cambridge, UK: Cambridge University Press.
- Streelman, J.T. and Danley, P.D. 2003. The stages of vertebrate evolutionary radiation. *Trends Ecol Evol*, **18**: 126-131.
- Svanbäck, R. and Persson, L. 2009. Population density fluctuations change the selection gradient in Eurasian perch. *Am Nat*, **173**: 507-516.
- Svanbäck, R., Pineda-Krch, M. and Doebeli, M. 2009. Fluctuating population dynamics promotes the evolution of phenotypic plasticity. *Am Nat*, **174**: 176-189.
- Svärdson, G. 1952. The coregonid problem. IV. The significance of scales and gillrakers. *Rep Inst Freshw Res Drott*, **33**: 204-232.
- Svärdson, G. 1957. The coregonid problem. VI. The palearctic species and their intergrades. *Rep Inst Freshw Res Drott*, **38**: 267-356.
- Svärdson, G. 1979. Speciation of Scandinavian *Coregonus*. *Rep Inst Freshw Res Drott*, **57**: 3-95.
- Taylor, E.B., Boughman, J.W., Groenenboom, M., Sniatynski, M., Schluter, D. and Gow, J.L. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol Ecol*, **15**: 343-355.
- Tobler, M., Riesch, R., Tobler, C.M., Schulz-Mirbach, T. and Plath, M. 2009. Natural and sexual selection against immigrants maintains differentiation among micro-allopatric populations. *J Evol Biol*, **22**: 2298-2304.
- Todd, T.N. 1998. Environmental modifications of gillraker number in coregonine fishes. *Advanc Limnol*, **50**: 305-315.
- Turgeon, J., Estoup, A. and Bernatchez, L. 1999. Species flock in the North American Great Lakes: molecular ecology of Lake Nipigon ciscoes (Teleostei: Coregonidae: *Coregonus*). *Evolution*, **53**: 1857-1871.
- Vamosi, S.M. 2003. The presence of other fish species affects speciation in threespine sticklebacks. *Evol Ecol Res*, **5**: 717-730.
- Vonlanthen, P., Bittner, D., Hudson, A.G., Young, K.A., Müller, R., Lundsgaard-Hansen, B., Roy, D., Di Piazza, S., Largiader, C.R. and Seehausen, O. 2012. Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature*, **482**: 357-363.

- Vonlanthen, P., Roy, D., Hudson, A.G., Largiader, C.R., Bittner, D. and Seehausen, O. 2009. Divergence along a steep ecological gradient in lake whitefish (*Coregonus* sp.). *J Evol Biol*, **22**: 498-514.
- Waddington, C.H. 1953. Genetic assimilation of an acquired character. *Evolution*, **7**: 118-126.
- West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst*, **20**: 249-278.
- West-Eberhard, M.J. 2003. *Developmental plasticity and evolution*. Oxford, UK: Oxford University Press.
- West-Eberhard, M.J. 2005. Developmental plasticity and the origin of species differences. *PNAS*, **102**: 6543-6549.
- Woods, P.J., Müller, R. and Seehausen, O. 2009. Intergenomic epistasis causes asynchronous hatch times in whitefish hybrids, but only when parental ecotypes differ. *J Evol Biol*, **22**: 2305-2319.
- Zimmerman, M.S., Krueger, C.C. and Eshenroder, R.L. 2006. Phenotypic diversity of lake trout in Great Slave Lake: differences in morphology, buoyancy, and habitat depth. *Trans Am Fish Soc*, **135**: 1056-1067.

Paper I

Paper II

Paper III

Paper IV

