Evolutionary Genetics of Atlantic Salmon (*Salmo salar* L.)

- Molecular Markers and Applications

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Doctoral thesis
Swedish University of Agricultural Sciences
Umeå 2004

Acta Universitatis Agriculturae Sueciae
Silvestria 324

ISSN 1401-6230
ISBN 91-576-6708-X

Tryck: SLU, Grafiska Enheten, Umeå, Sweden, 2004

Abstract

Vasemägi, A. 2004. *Evolutionary Genetics of Atlantic salmon (Salmo salar L.) - Molecular Markers and Applications.*
ISSN 1401-6230, ISBN 91-576-6708-X

This thesis deals with evolutionary genetics of Atlantic salmon populations, with the special emphasis on the roles of migration, random genetic drift, mutation and natural selection affecting the patterns of molecular variation across contemporary and historical time scales. Studies of mitochondrial DNA variation supported the hypothesis of multiple post-glacial colonization events of the Baltic Sea. The Eastern Atlantic populations differ from the geographically close southern Baltic populations, indicating absence of inward and limited outward gene flow through the Danish straits during the last 8000 years. Four common European mitochondrial haplotypes derive from the ancestral ND1-BBBA haplotype by one-step substitutions. Our results suggest that wild populations have an important role in re-colonization processes of the former salmon rivers where populations have been driven to extinction due to human activities. Spatio-temporal analysis over eighteen years provided genetic evidence of immigration from compensatory hatchery releases into one of the biggest wild Atlantic salmon population in the Gulf of Bothnia (R. Vindelälven) and emphasize the genetic risks associated with current large-scale stocking practices in the Baltic Sea. For restoration of former salmon rivers in the Gulf of Finland we recommend that two closest native salmon populations should be preferred to help to fill in the currently missing "building blocks" that are important for the persistence of genetic variation and long-term survival of salmon populations in Estonia. We identified several expressed sequence tag (EST) loci that are potentially affected by divergent selection demonstrating that EST-scans may provide suitable strategy to discover functionally important genetic variation both in model and non-model organisms.

Keywords: population genetics, microsatellites, mitochondrial DNA, MHC, adaptation, natural selection, migration, genetic drift, expressed sequence tags, EST

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Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., Kohlmann, K., Löytynoja, A., Nielsen, E.E., Paaver, T., Primmer, CR., Titov, S., Vasemägi, A., Veselov, A., Öst, T. & Lumme, J. 2001. Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonisation of the Baltic Sea area. *Molecular Ecology*, 1, 89-102.

II. Vasemägi, A., Gross, R., Paaver, T., Kangur, M., Nilsson, J. & Eriksson, L-O. 2001. Identification of the origin of Atlantic salmon (*Salmo salar* L.) population in a recently recolonized river in the Baltic Sea. *Molecular Ecology*, 10, 2877-2882.

III. Vasemägi, A., Gross, R., Paaver, T., Koljonen, M-L. & Nilsson, J. Caught at scene: extensive immigration from compensatory hatchery releases into wild Atlantic salmon population in the Baltic Sea. *Submitted*.

IV. Vasemägi, A., Gross, R., Paaver, T., Koljonen, M-L., Säisä, M. & Nilsson, J. Analysis of gene associated tandem repeat markers in Atlantic salmon (*Salmo salar* L.) populations: implications for restoration and conservation in the Baltic Sea. Accepted in *Conservation Genetics*.

V. Vasemägi, A., Nilsson, J. & Primmer, C.R. Expressed sequence tag (EST) linked microsatellites as a source of gene associated polymorphisms for detecting signatures of divergent selection in Atlantic salmon (*Salmo salar* L.). *Submitted*.

Introduction

"*For the evolutionist, differences are what evolution is all about and are expected, whereas persistent similarities across organisms need to be explained*."
Lewontin (2002)

Interpretation of DNA variation

Evolution can be defined as a change in allele frequencies over time. A fundamental goal of evolutionary biology is to better understand how natural selection operates in interaction with other evolutionary processes such as mutation, migration and random genetic drift. Determination of the relative roles of single evolutionary forces that affect the genetic variation across genomes or populations is a challenging task, particularly because researchers usually depend on *static* data, observations of the patterns of genetic variation within and between populations or species, to infer the *dynamic* processes that could not be directly observed (Lewontin, 2002). However, very rapid growth of sequence information in broad range of organisms, including salmonids (Bayne *et al*., 2001; Davey *et al*., 2001; Thorgaard *et al*., 2002; Martin *et al.*, 2002; Rise *et al.*, 2004; Tsoi *et al.*, 2004) and advances in statistical methods (Rousset & Raymond 1997; Luikart & England 1999; Yang & Bielawski 2000; Beaumont & Rannala 2004) offer an exciting possibility to evaluate the relative roles of different evolutionary factors. Furthermore, the possibility to study DNA from 'historical' or 'ancient' material, such as bones and scales (Nielsen, Hansen & Loeschcke, 1997; Nielsen, Hansen & Loeschcke, 1999; Hansen *et al.*, 2002; Consuegra *et al.*, 2002; Hutchinson *et al.*, 2003; Brzuzan *et al.*, 2004; Wang, Cannon & Saunders 2004), enables addition of a temporal dimension to population genetic research and thus, makes it possible to study evolution as a *dynamic* process (Stockwell, Hendry & Kinnison 2003).

Molecular markers

The double-helical structure of deoxyribonucleic acid (DNA) molecule was deduced fifty years ago by Watson and Crick (1953) who stated: "*This structure has novel features which are of considerable biological interest*". The main breakthrough in molecular biology, however, was driven by the invention of polymerase chain reaction (PCR) about 20 years ago (Saiki, Scharf & Faloona, 1985). Since then, the use of molecular markers for revealing polymorphism at DNA level, has been playing an increasing role in ecological and evolutionary research (Sunnucks, 2000). Among others, the mitochondrial DNA (mtDNA) and microsatellite loci have been the most widely used markers promoting the 'DNA revolution' (Cavalli-Sforza, 1998).

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is considered the classical and one of the most suitable marker type for phylogeographical studies for several reasons (Avise, 1994). Particularly, mtDNA is haploid, maternally inherited, non-recombining and usually considered as selectively neutral marker. The mtDNA molecule is effectively a single locus, where each haplotype is equivalent to allele. Because mtDNA is maternally inherited and haploid, it is affected more by random genetic drift than nuclear loci. Hence, populations tend to be more different from each other at mtDNA than nuclear loci, especially when the migration is male-biased (i.e. males tend to migrate more; Frazer, Lippe & Bernatchez 2004; Bekkevold, Hansen & Mensberg 2004).

Mini- and microsatellites

Micro- and minisatellites are simple tandemly repeated DNA sequence motifs 1-6 and 10-100 basepairs long, respectively and are common throughout the nuclear genomes of eukaryotes (Jarne & Lagoda 1996). They consist of a short sequence motif, such as 'CA-CA-CA-CA' or 'GTTATTAAAT-GTTATTAAAT' that are often repeated a variable number of times. The advantage of these tandem repeat markers stems for their very high variability which enables to efficiently apply them both at individual and population level analysis (e.g. Waser & Strobeck 1998; Manel *et al.*, 2003; but see Hedrick, 1999; Balloux *et al*., 2000; Moss, Piertney & Palmer, 2003 for a cautionary perspective). Usually, tandem repeat markers are considered as evolutionarily neutral DNA markers (but see Li *et al*., 2002 for an alternative view). However, selection can affect the nearby flanking neutral variation, known as genetic hitchhiking (Maynard-Smith & Haig 1974).

Atlantic salmon

The Atlantic salmon (*Salmo salar* L.) is highly valuable fish species due to its importance in fisheries, recreational angling and aquaculture, and is naturally distributed along the east and west coast of the North Atlantic Ocean where it exists in both anadromous (migratory) and landlocked (freshwater) forms. Together with the rainbow trout (*Oncorhynchus mykiss*) it is one of the most intensively studied fish species in a wide range of research areas (Thorgaard *et al.*, 2002; Rise *et al*., 2004). As most salmonid species, Atlantic salmon is divided into geographically distinct units that are more or less isolated from each other. Numerous studies have demonstrated that Atlantic salmon forms distinct populations on river or tributary basis (McConnell *et al*., 1995; Sanchez *et al*., 1996; Koljonen *et al*., 1999; Verspoor *et al*., 1999; Spidle *et al*., 2001; King *et al* 2001). Within Europe, Eastern Atlantic and Baltic populations form distinct groups (Nilsson, 1997; Verspoor *et al*., 1999). The deepest divergence has been found between the North American and European populations (King *et al*., 2001; Nilsson *et al*., 2001; Koljonen *et al*., 2002; Wennevik *et al*., 2004).

Phylogeographic inference – interpreting the present to understand the past

Populations are affected not only by ongoing evolutionary processes but also by historical events like glacial cycles which have considerably affected the distribution of organisms in Europe and Northern America resulting in massive extinction-colonization events (Hewitt, 1996). Phylogeography investigates the geographical distribution of genealogical lineages (Avise, 1994) and may provide snapshots of evolutionary history. It is important to remember that current phylogeographic patterns represent only a *static* information from a single time point and may result in misleading evolutionary implications, as demonstrated by the mtDNA analysis of 16 000 to 40 000 year old Atlantic salmon (Consuegra *et al.*, 2002) and 14000 to 42000 year old brown bear specimens (Leonard, Wayne & Cooper, 2000). Nevertheless, phylogeographic studies over large geographical areas are essential to understand the overall level of structuring of particular species.

Population Genetics/Genomics – interacting evolutionary forces

Numerous genetic studies have described the distinctiveness of salmonid populations at different spatial scales. However, based on patterns of variation at genetic markers inferences can also be done regarding effective population size (e.g. Hansen *et al.*, 2002; Heath *et al.*, 2002; Laikre *et al.*, 2002), life history (e.g. Hansen *et al.*, 2000), behaviour (e.g. Tiira *et al.*, 2003), relatedness (e.g. Fontaine & Dodson 1999) and adaptation (Landry *et al.*, 2001; Koskinen, Haugen & Primmer, 2002; Campbell & Bernatchez 2004). Furthermore, the possibility to apply genome-wide analysis in increasing number of organisms enables to distinguish evolutionary processes that act on individual loci (i.e. mutation, selection) from the whole genome effects (i.e. genetic drift, migration) (Fig. 1; Black *et al*., 2001; Luikart *et al*., 2003).

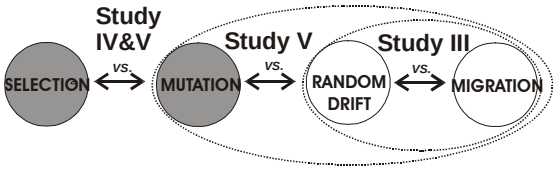

Figure 1. Schematic illustration of the various tests applied in the thesis to investigate the relative roles of different evolutionary forces affecting allele frequency distributions of studied salmon populations. Locus-specific and genome wide processes are shown with grey and white colours, respectively.

Conservation Genetics – looking forward

The fact that genetics can give invaluable information for more effective and biologically sound management of endangered species has lead to increasing number of studies in the field of conservation genetics. To date, most of the investigations have employed neutral genetic variation (i.e markers from non-coding parts of the genome) largely because of the limited knowledge about the

genes that influence the fitness and survival in natural environments (Hedrick, 2001; van Tienderen *et al.*, 2002).

Currently, 80-90% of the Atlantic salmon in the Baltic Sea originates from hatcheries (ICES, 2003) and many wild Atlantic salmon populations are on the brink of extinction, while the production of farmed salmon in the North Atlantic is 300 times greater than the annual catch of wild salmon (WWF, 2001). Consequently, the erosion of the natural gene pools through interbreeding with farmed fish has been recognized as one of the major threat to wild Atlantic salmon populations (WWF, 2001). It follows that genetics can play an important role in conservation and restoration of the wild salmon populations.

Objectives

In this thesis the Atlantic salmon is used as a model species to evaluate the relative roles of migration, random genetic drift, mutation and natural selection affecting the patterns of genetic variation within and between populations at different spatial scales, and across contemporary and historical time scales.

The main objectives of this thesis were:

1. to reveal population structuring in Northern Europe and infer the potential postglacial colonization routes of the Baltic Sea area by means of mtDNA variation (**I**).

2. to identify the origin of founders of recently recolonized river in the Baltic Sea where the native population has been driven to extinction (**II**).

3. to determine the origin of presumably non-native fin-damaged fish caught in the River Ume/Vindelälven and estimate the rate of immigration and potential impact of straying on the wild R. Vindelälven salmon population during the time period from 1985 to 2003 (**III**).

4. to evaluate the relative importance of selection versus neutral evolutionary forces in shaping allele frequencies at eight genomic microsatellites and six gene associated tandem repeat markers among nine wild and hatchery populations in the Baltic Sea (**IV**).

5. to search for the genetic signatures of selection by screening 17 genomic and 79 expressed sequence tag (EST) associated mini- and microsatellites in eight wild salmon populations inhabiting contrasting natural environments (salt-, brackish- and freshwater habitat) (**V**).

6. to test at which spatial scale stepwise-like mutations have contributed to the genetic differentiation at 96 tandem repeat markers in eight salmon populations from Northern Europe (**V**).

Materials and Methods

Studied Atlantic salmon populations were sampled from different geographical scales, with special emphasis on the Baltic salmon populations (Fig. 2A). Detailed maps can be found in the original papers. As a result of technical developments in molecular analyses of historical material such as from old scale material, the temporal dimension of studies of the thesis span from five (**II**) to eighteen years (**III**). Different marker types (mtDNA, mini- and microsatellites) were applied separately (**I**, **II**) or simultaneously (**III**, **IV**, **V**) for investigating different evolutionary aspects in Atlantic salmon. Recent development of molecular technology has considerably increased the genotyping efficiency. As a result, manual electrophoresis and genotyping procedures used partly in the study **III** and **IV** (see Fig. 2B for a 'classical' gel image) have been replaced to a great extent by the use of automatic sequencers (study **II** and **V**). This thesis also reflects the rapid growth of sequence information in salmonids arising from initiated genome projects (Genomics Research on Atlantic Salmon Project, Canada; Norwegian Salmon Genome Project) and developments of new analytical approaches. In particular, statistical methods for estimating immigration rates, genetic signatures of selection and the role of mutations in population divergence have been developed only very recently (Kauer, Dieringer & Schlötterer, 2003; Hardy *et al*., 2003; Wang & Whitlock 2003; Beaumont & Balding 2004). As such, the studies **III**, **IV** and **V** would not be feasible to conduct just 2-3 years earlier. Detailed laboratory procedures and applied analytical approaches are specified in the original papers.

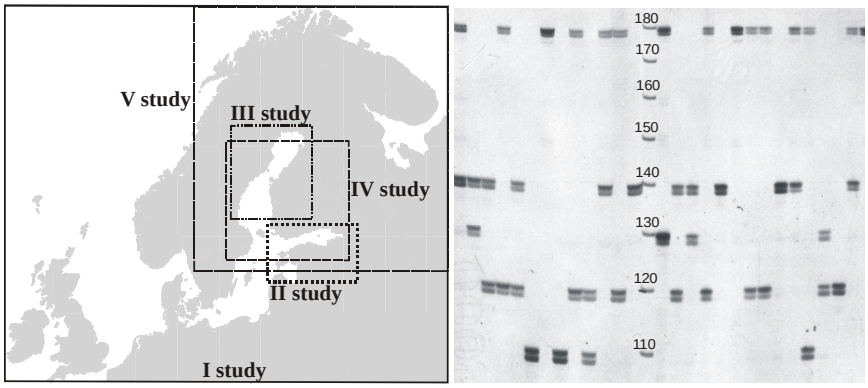

Figure 2. A) Map of Northern Europe showing the approximate spatial scales of the studies. B) Banding patterns of the major histocompatibility complex class II alpha chain (*MHCIIα*) linked minisatellite marker after electrophoresis in 8% denaturing polyacrylamide gel and silver staining from the study **IV**.

Results –Summary of Papers

Paper I. This study aimed at revealing population structuring and evaluating phylogenetic relationships among 46 Atlantic salmon populations in Europe by using PCR fragment length polymorphism (PCR-RFLP) analysis (Fig. 3). In addition, RFLP haplotypes from different parts of the distribution area were sequenced and the phylogeny of European haplotypes and their relations to the North American lineage was described (Fig. 4). The four common European haplotypes derive from the ancestral ND1-BBBA (rooting the European clade to the North American) by one-step substitutions: AAAA < AABA < BBBA > BBBB. The Swedish west-coast populations differ from the geographically close southern Baltic, indicating absence of inward and limited outward gene flow through the Danish straits during the last 8000 years. Within the Baltic Sea, only three ND1 haplotypes were detected and there was no variation for ND3/4/5/6. In the whole southern Baltic and in lakes Vänern, Ladoga and Onega the haplotype AABA dominated.

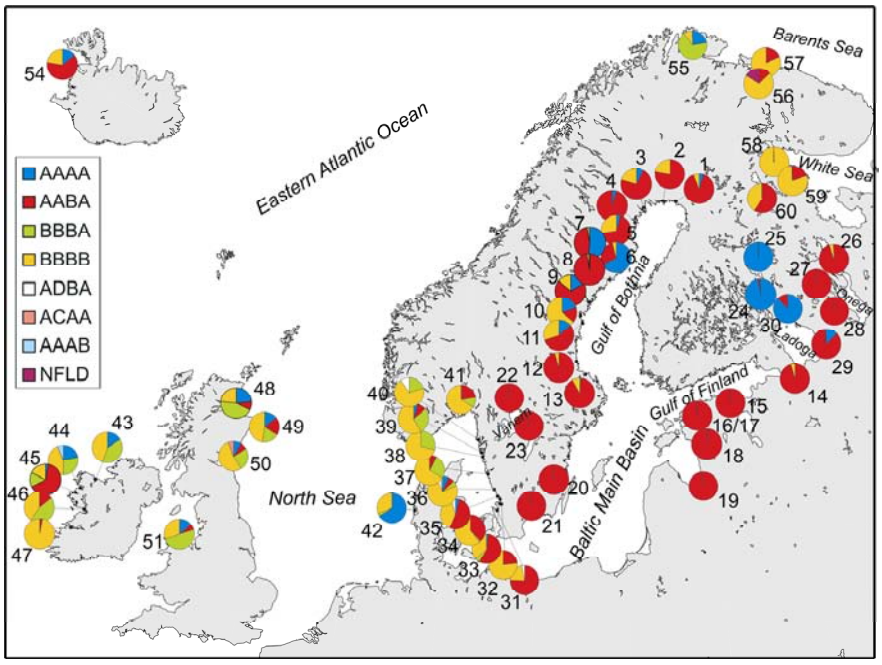

Figure 3. Map of sampling locations with pie diagrams showing the distribution of haplotype frequencies among studied Atlantic salmon populations. Earlier published RFLP data on 726 specimens from 17 populations (Palva, Lehväslaiho & Palva, 1989; Nielsen, Hansen & Loeschke, 1996; Verspoor *et al*., 1999) are included into the map.

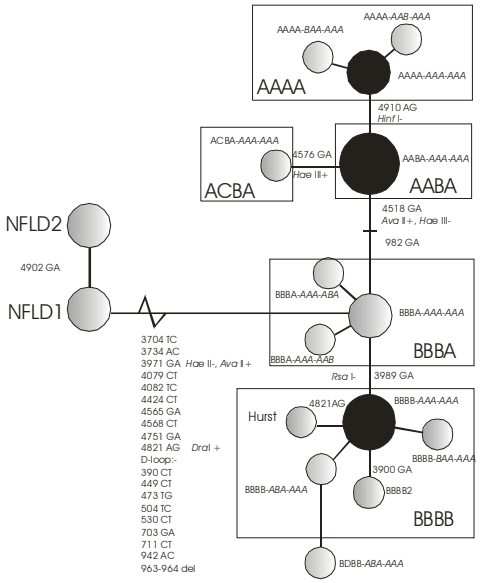

Figure 4. Phylogeny of the mitochondrial types as a minimum spanning network. The nucleotide sequence information on D-loop (Kauppi *et al.*, 1997), 16S rRNA, tRNA-leu, ND1 and tRNA-ile is used to infer the structure of the network and the variable restriction sites are marked. The three haplotypes found in the Baltic area are shown in black color. The derived haplotypes reported in Nielsen, Hansen & Mensberg (1998) on the basis of additional variation in ND3/4 and ND5/6 are also marked.

Paper II. The main objective of this study was to investigate the founder event in a recently recolonized salmon population in Gulf of Finland, Baltic Sea (R. Selja). To identify the origin of the founders, four geographically closest wild populations and two hatchery stocks were analysed using six microsatellite loci. The results of assignment tests and factorial correspondence analysis indicate that the initial recolonizers of the river Selja originated from the geographically nearest (7 km) wild population (R. Kunda). Additionally, interbreeding (hybridization) between recolonizers and stocked hatchery individuals was detected in subsequent years. Interbreeding between the recolonizers and the hatchery individuals was not directly evident from the individual assignments, but visualization of the multilocus genotypes using factorial correspondence analysis indicated the probable occurrence of first-generation hybrids between wild colonizers and hatchery fish.

Paper III. In this study we assessed the rate and impact of immigration from main hatchery stocks of Gulf of Bothnia into one of the largest wild salmon populations in the Baltic Sea, River Vindelälven, within a temporal framework of eighteen years (Fig. 5).

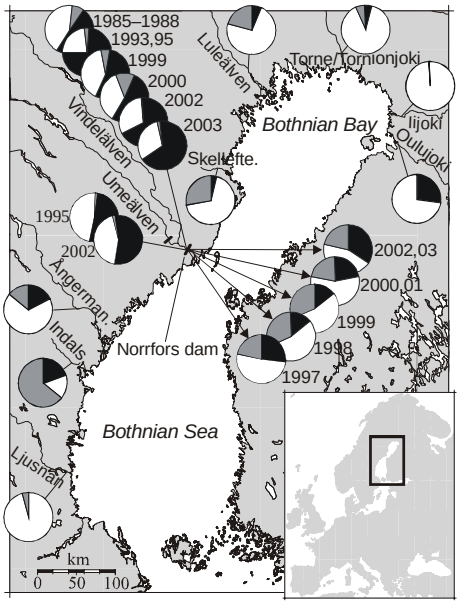

Figure 1. Map of sampling locations with pie diagrams showing the spatio-temporal distribution of mtDNA haplotype frequencies among studied Atlantic salmon populations. Haplotype frequencies of the suspected immigrants with damaged fins caught below the Norrfors dam in the R. Ume/Vindelälven are indicated by arrows. The haplotypes AABA, BBBB and AAAA are designated by white, grey and black color, respectively.

Using mixed stock analysis we provide genetic evidence based on mtDNA and microsatellite markers that a large proportion (66%) of suspected immigrants caught in the R. Ume/Vindelälven during 1997-2003 originated from R. Ångermanälven, R. Luleälven and R. Ljusnan hatcheries (Fig. 6). The estimated contribution of fin damages fishes from five other hatcheries was not significantly different from zero. Maximum-likelihood estimate of immigration rate from these hatcheries into the wild R. Vindelälven population was 0.068 (95%CI 0.021-0.128) over the studied time period and reached up to a quarter (m=0.249, 95%CI 0.106-0.419) of the total population during 1993-2000 (Fig. 7). Immigration rate from five other non-native hatcheries into the wild R. Vindelälven population was marginal and not significantly different from zero (m=0.01, 95%CI 0-0.047). As expected, significant decline in F_{ST} estimates between temporal samples of wild R. Vindelälven population and hatchery stocks of rivers Luleälven and Ångermanälven that contributed the majority of the strayers was observed. Simulations using different unidirectional migration rates from hatchery stocks of Ångermanälven, Luleälven and Ljusnan (with 4:2:1 proportion, respectively) into the wild R. Vindelälven population showed the sharpest decrease in F_{ST} values after the first generation and relatively slow decline in F_{ST} when $m \leq 0.07$.

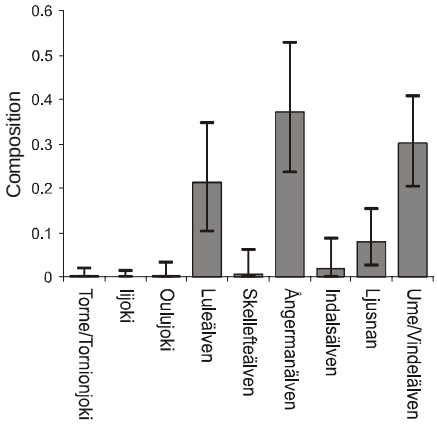

Figure 6. Mean proportions of hatchery contributions among suspected immigrants (n=181) caught in the River Ume/Vindelälven during 1997-2003 as revealed by mixed stock analysis (95% posterior probability interval shown as whiskers).

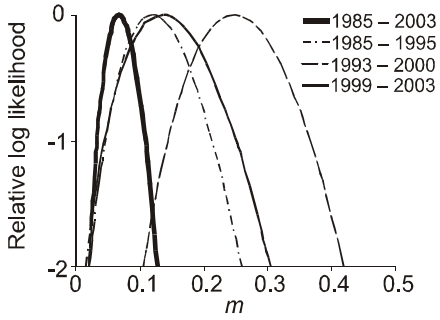

Figure 7. Relative log-likelihood curves of estimated immigration rates from the hatchery stocks of rivers Ångermanälven, Luleälven and Ljusnan into the wild R. Vindelälven population during the period from 1985 to 2003.

Paper IV. The first aim of this study was to compare the patterns of genetic diversity and differentiation among nine salmon populations in the Baltic Sea based on eight genomic and six gene-associated mini- and microsatellite markers in order to evaluate the relative importance of selection versus neutral forces in shaping allele frequencies at these markers. The simulation analyses indicated that two gene linked markers (*MHCIIa*, *ARP*) and genomic microsatellite *Ssa171* exhibited significant departures ($P<0.05$) from the neutral expectations.

Secondly, we combined the information from both types of markers in order to suggest the most suitable donor populations for restoration of salmon populations in three former salmon rivers in the Gulf of Finland. In contrast to other studies which have compared wild and reared fish stocks (e.g. Clifford, McGinnity & Ferguson 1998; Koljonen *et al.*, 1999, Norris, Bradley & Cunningham, 1999), we found more genetic variation in hatchery stocks than in the wild salmon populations from the Gulf of Finland (Fig. 8). The total number of alleles found in R. Loobu 1999 year-class was 29% smaller than in 1996 year-class (39 and 55

alleles, respectively) in spite of a larger sample in 1999, suggesting that only limited number of breeders have contributed to this cohort. Nevertheless, all three wild salmon populations in Gulf of Finland possessed alleles at several gene-associated markers (e.g. *MHCI* and *IGF*) that were not detected in two more variable hatchery stocks.

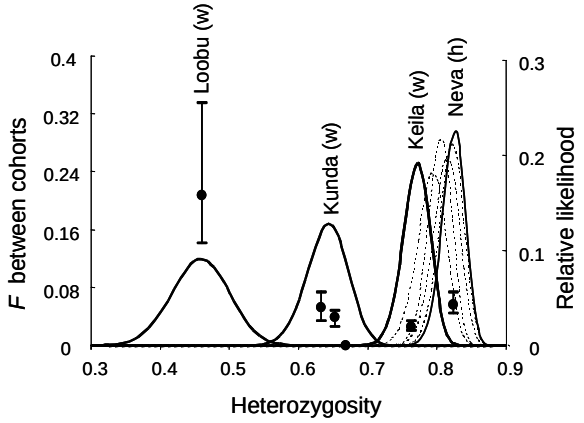

Figure 8. Temporal variation (*F*) between the year-classes (cohorts) of populations shown as black bullets (95% confidence intervals shown as whiskers) and likelihood curves of heterozygosity estimates for each population. Continuous curves correspond to four Gulf of Finland populations (from left to right: R. Loobu, R. Kunda, R. Keila populations and R. Neva stock). Broken curves represent populations with only single temporal sample available. The highest peak of the curve corresponds to population maximum likelihood value of estimated heterozygosity. Wild and hatchery populations are designated with the letters (w) and (h), respectively.

Paper V. In this study we searched for the genetic signatures of divergent selection by screening 17 genomic and 79 expressed sequence tag (EST) derived mini- and microsatellites in eight natural Atlantic salmon populations inhabiting contrasting natural environments (salt, brackish and freshwater habitat). Gene diversity and number of alleles differed significantly (Wilcoxon's signed rank test: gene diversity $P<0.05$; number of alleles, $P<0.001$) among populations from salt-, brackish and freshwater habitats. Several EST associated microsatellites exhibit highly significant deviations from the neutral expectations using different statistical methods at various spatial scales (small spatial scale, Fig. 9). At large spatial scale stepwise-like mutations contributed significantly to the observed microsatellite divergence (observed R_{ST}>permuted R_{ST}).

re-colonization can occur on the basis on nearby rivers when the environmental conditions become suitable. Study **III** indicated that current large-scale hatchery release practices may have increased straying rate beyond the 'natural' level in the Baltic Sea. Study **IV** emphasizes the importance of random genetic drift in small salmon populations. Both study **IV** and **V** demonstrated that selection may have important role in shaping allele frequencies both in some genomic and gene associated markers. Additionally, the study **V** revealed that stepwise-like mutations have contributed to the genetic divergence at tandem-repeat markers at large geographical scale.

Specific conclusions, findings and recommendations from the present thesis are:

Paper I. Three hypotheses of the origin of Baltic salmon have previously been proposed: i) from the eastern refugia in pre-glacial lakes (Kazakov & Titov 1991); ii) from the western Atlantic populations at the beginning of the Yoldia stage through the Närke strait (Verspoor *et al*., 1999); iii) from both directions (Koljonen *et al*., 1999). The results of mtDNA analyses were consistent with the earlier allozyme study of Ståhl (1987) which demonstrated clear separation of the Baltic and Eastern Atlantic salmon populations and support the hypothesis of postglacial colonization of the Baltic Sea by multiple lineages (Koljonen *et al*., 1999). Genetic database of the Baltic salmon populations consisting of mtDNA and microsatellite genotypes (Säisä *et al*., submitted) will serve as a valuable baseline for further applications using individual assignments and/or mixed-stock analyses.

Paper II. Our results suggest that the native populations still have an important role in colonization processes of the former salmon rivers although, the hatchery releases are outnumbering the wild salmon recruitment in the Baltic Sea at present. Therefore, stocking activities should not be considered as an essential prerequisite for re-establishment of former salmon rivers when adjacent wild salmon population(s) still exist. Identification of the wild Kunda population as the most likely source of the recolonizers is surprising considering that the proportion of hatchery releases compared to wild production is even more pronounced (approximately 37: 1) in the Gulf of Finland (ICES 1998), compared to the total Baltic where the stocked fish outnumber the wild salmon approximately eight- or nine-fold. Surprisingly fast re-colonization of the extinct river may indicate a higher level of straying to the unoccupied habitats than the present gene flow between extant salmon populations. Rapid colonization of the new areas, followed by smaller straying rates after the populations have become established, has been described in several Pacific salmon (*Oncorhynchus*) species (Quinn, 1993).

Paper III. This study provides genetic evidence of immigration from compensatory hatchery releases into wild Atlantic salmon population in the Gulf of Bothnia (R. Vindelälven) and emphasizes the genetic risks associated with current large-scale stocking practices in the Baltic Sea. Detection of R. Luleälven hatchery as one of the major immigrant sources was not unexpected for several reasons. First, R. Luleälven hatchery production (over half a million smolts per year) is the second largest in the Baltic Sea. Second, R. Luleälven hatchery stock has been formed by mixing salmon from different origin (from rivers Luleälven,

Ångermanälven, Skellefteälven, Indalsälven and Ume/Vindelälven). Candy & Beacham (2000) showed that artificially made hybrid stocks of chinook salmon (*Oncorhynchus tshawytscha*) exhibited three times higher straying rate than the native stock if released at the same time and location, suggesting that hybridization could lead to elevated amount of straying. The amount of hatchery releases into the R. Ångermanälven is around 200 000 smolts per year, while the third immigrant source, R. Ljusnan hatchery, releases approximately 185 000 smolts per year but being more distant from R. Ume/Vindelälven it contributed only a small proportion of the findamaged immigrants.

Elevated immigration into R. Ume/Vindelälven during the period from 1993 to 2000 raises the obvious question why this happened during this period given that large-scale hatchery releases have been carried out already more than forty years in the Baltic. As one possible hypothesis, we suggest that increased genetic impact of compensatory releases on the wild populations could be associated with the outbreak of M74 disease syndrome in the Baltic during 1990´s (Hansson *et al*., 2001). Estimated M74 mortality among salmon fry in the Gulf of Bothnia populations has been the highest during 1992-1996 ranging from ca 50 to 90% (ICES 2003). The M74 is associated with the low levels of vitamin B_1 (thiamine) in salmon eggs and fry and hatcheries have routinely used thiamine treatment of fry since 1996 to prevent the development of M74 syndrome. As a result, the production of hatchery-reared salmon has stayed high and relatively stable while the effect of M74 in the wild populations has been substantial. In the R. Vindelälven, for example, the lowest parr density estimates have been recorded during the years of high M74 incidence. Therefore, M74 might be responsible for changing the ratio between breeders of wild and hatchery origin and hence, cause increased genetic impact of compensatory releases on wild populations during the second half of the 1990`s.

Paper IV. Lower than expected divergence at the major histocompatibility complex linked marker (*MHCIIa*) may result from balancing selection, while the genomic microsatellite *Ssa171* might be affected by directional selection. More homogeneous allelic distribution at the *MHCIIa* linked minisatellite than observed at other loci and obtained from coalescent simulations could be caused by similar pathogenic pressures at common feeding grounds in the sea. For example, vaccination experiments have shown that bacterial pathogens *Aeromonas salmonicida*, *Vibrio anguillarum* and *Yersinia ruckeri* are playing important role in survival of Atlantic salmon in the Baltic Sea (Buchmann, Larsen & Therkildsen 2001). Differentiation pattern at *MHCI* associated microsatellite on the other hand was consistent with the neutral expectations and there was no need to invoke selective forces to explain the observed relationships among populations. This might suggest that viral pathogens have smaller impact on the *MHCI* variation in contemporary populations compared to the neutral evolutionary forces.

Loss of genetic variation in reared stocks in comparison with wild populations of Atlantic salmon has been reported in several studies (e.g. Clifford, McGinnity & Ferguson 1998; Koljonen *et al*., 1999; Norris, Bradley & Cunningham, 1999). In contrast, we found more genetic variation within two hatchery stocks than in wild populations of Gulf of Finland. Several factors can contribute to this unusual

phenomenon. The studied Estonian wild salmon populations are rather small – the estimated annual smolt production ranges from 500 (rivers Keila and Loobu) to 900 (R. Kunda) and electrofishing surveys have revealed very weak or even missing year-classes in all three populations during the last decade (Kangur & Viilmann 2001). In contrast, the historical R. Neva salmon population has been historically much larger and hence, should be also more variable. Maintenance of the high genetic diversity of the R. Neva hatchery stock (founded in the 1970s; Kallio 1986) may then indicate that adequate breeding practices have been carried out during the captive breeding programme. High diversity of the R. Narva hatchery stock, however, may be at least partly caused by its mixed genetic origin (mixture of R. Neva and Latvian salmon populations). We suggest that distinct ecological conditions, the presence of alleles not found in hatchery stocks and moderate genetic differentiation between the wild and hatchery stocks (F_{ST}=0.055-0.187) justifies conservation efforts of these last remaining wild salmon populations of Gulf of Finland. We also recommend that two closest native salmon populations (R. Keila and R. Kunda) should be preferred over the hatchery stocks for restoration of former salmon rivers in Estonia to help to fill in the currently missing "building blocks" that are essential for the persistence of genetic variation and long-term survival of salmon populations in Estonia.

Paper V. We identified several EST loci that are potentially affected by divergent selection at various spatial and environmental scales and hence, they serve as promising genes that are associated with the 'local' adaptation in Atlantic salmon. As emphasized in earlier studies, significant results with more than one neutrality test only raises the candidate status of particular locus but does not demonstrate selection *per se* (e.g. Schlötterer 2002; Vigorouroux et al. 2002; Campell and Bernatchez 2004). Therefore, the identified candidate EST loci will serve as a basis for further sequence analysis to validate the role of divergent selection in these genes. To our knowledge, evidence of divergent selection among contemporary wild Atlantic salmon populations has been reported only at two genes (*MHCIIβ*, Landry and Bernatchez 2001; *MEP-2*, Verspoor & Jordan 1989).

It is likely that inconsistent results of one of the neutrality test (*F* test; Vitalis, Dawson & Boursot, 2001) compared to other methods at large spatial scale were in large part caused by stepwise-like mutations which occurred after the population divergence, as indicated by allele permutation test of Hardy *et al.*, (2003). Apparent discrepancy between population-specific divergence test (Vitalis, Dawson & Boursot, 2001) and other methods suggests that the deviations from the neutrality of *F* test must be taken with considerable caution when mutations in tandem repeat markers have contributed to the population divergence. Because of the inherent difficulties of multiple statistical testing related to genome-scans, a practical approach would be to simultaneously apply two or more neutrality tests which are based on different assumptions and parameter estimation (e.g. Storz, Paseur, and Nachman 2004) and select for the next validation step (sequence analysis or/and QTL mapping) outlier loci that are supported by several methods.

Close evolutionary relationships and successful cross-amplification results between genera *Salmo* and *Oncorhynchus* (Rexroad & Palti 2003; Rise *et al.*,

2004) indicate that Atlantic salmon and rainbow trout EST-derived microsatellites can be easily applied for genome-scans in other species within the subfamily Salmoninae. Although in the present study we concentrated on EST-linked microsatellites, other marker types like indels and SNPs can be also quickly developed from the available EST sequences and successfully applied for screening the signatures of selection, particularly among closely related populations. In the light of encouraging simulations by Beaumont and Balding (2004), EST-scans may provide suitable strategy to discover functionally important genetic variation both in model and non-model organisms. Also, given the relative ease of conducting large-scale multi-locus screens for natural selection (Wilding, Butlin & Grahame, 2001; Campell & Bernatchez 2004) it is likely that more emphasis will be directed to outlier verification and characterization in the future.

Future perspectives

Recent developments in high through-put DNA technologies provide for the first time an opportunity to expand the types of analyses that have previously been addressed in humans and few model organisms to other (more interesting!) species such as salmonid fishes. It is likely that the fast development of genomic and statistical approaches will provide new insights to the adaptation processes at molecular level in natural populations and environments thereby enabling a greater understanding of the interactions between molecular processes, evolution and ecosystem biology. Increased knowledge about the function of Atlantic salmon genes will provide valuable information for conservation efforts of wild populations and basis for further development of salmon aquaculture industry. Emerging new disciplines with the *–omics* suffixes will likely answer many unresolved questions and provoke new challenges.

References

Avise J.C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York, USA.

Balloux. F., Brunner, H., Lugon-Moulin, N., Hausser, J. & Goudet, J. 2000. Microsatellites can be misleading: An empirical and simulation study. *Evolution* 54, 1414-1422.

Bayne, C.J., Gerwick, L., Fujiki, K., Nakao, M. & Yano, T. 2001. Immune-relevant (including acute phase) genes identified in the livers of rainbow trout, *Oncorhynchus mykiss*, by means of suppression subtractive hybridization. *Developmental and Comparative Immunology* 25, 205-217.

Beaumont, M.A. & Balding, D.J. 2004. Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology* 13, 969–980.

Beaumont, M.A. & Rannala, B. 2004. The Bayesian revolution in genetics. *Nature Reviews. Genetics* 5, 251-261.

Bekkevold, D., Hansen, M.M. & Mensberg, K-L.D. 2004. Genetic detection of sex-specific dispersal in historical and contemporary populations of anadromous brown trout *Salmo trutta. Molecular Ecology*, 13,1707-1712.

Black, W.C., Baer, C.F., Antolin, M.F. & DuTeau, N.M. 2001. Population genomics: Genome-wide sampling of insect populations. *Annual Review of Entomology* 46, 441-469.

Brzuzan, P., Ciesielski, S., Kusznierz, J. & Jurczyk, L. 2004. Identification of whitefish remains by mtDNA analysis. *Annales of Zoology Fennici* 41, 61–68.

Buchmann, K., Larsen, J.L. & Therkildsen, B. 2001. Improved recapture rate of vaccinated sea-ranched Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 24, 245-248.

Campbell, D. & Bernatchez, L. 2004. Genomic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution* 21, 45–956.

Candy, J.R. & Beacham, T.D. 2000. Patterns of homing and straying in southern British Columbia coded-wire tagged chinook salmon (*Oncorhynchus tshawytscha*) populations. *Fisheries Research* 47, 41-56.

Cavalli-Sforza L.L. 1998. The DNA revolution in population genetics. *Trends in Genetics* 14, 60-65.

Consuegra, S., De Leaniz, C.G., Serdio, A., Morales, M.G., Straus, L.G., Knox, D. & Verspoor, E. 2002. Mitochondrial DNA variation in Pleistocene and modern Atlantic salmon from the Iberian glacial refugium. *Molecular Ecology* 11, 2037-2048.

Clifford, S.L., McGinnity, P. & Ferguson, A. 1998. Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. *Journal of Fish Biology* 52, 118-127.

Davey, G.C., Caplice, N.C., Martin, S.A. & Powell, R. 2001. A survey of genes in the Atlantic salmon (*Salmo salar*) as identified by expressed sequence tags. *Gene* 263, 121-130.

Fontaine, P.M. & Dodson, J.J. 1999. An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites. *Molecular Ecology* 8, 189-198.

Frazer, D.J., Lippe, C. & Bernatchez, L. 2004. Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal on population structure in brook charr (*Salvelinus fontinalis*). *Molecular Ecology* 13, 67–80.

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E. & Mensberg, K.L.D. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology* 9, 583-594.

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E., Bekkevold, D. & Mensberg, K.L.D. 2002. Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology* 11, 2523-2535.

Hansson, S., Karlsson, L., Ikonen, E., Christensen, O., Mitans, A., Uzars, D., Petersson, E. & Ragnarsson, B. 2001. Stomach analyses of Baltic salmon from 1959–1962 and 1994–1997: possible relations between diet and yolk-sac-fry mortality (M74). *Journal of Fish Biology* 58, 1730-1745.

Hardy, O.J., Charbonnel, N., Fréville, H. & Heuert, M. 2003. Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics* 163:1467–1482.

Heath, D.D., Busch, C., Kelly, J. & Atagi, D.Y. 2002- Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Molecular Ecology* 11, 197-214.

Hedrick, P.W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. *Evolution* 53, 313-318.

Hedrick, P.W. 2001. Conservation genetics: where are we now? *Trends in Ecology & Evolution* 16, 629-636.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 58, 247-276.

Hutchinson, W.F., van Oosterhout, C., Rogers, S.I. & Carvalho, G.R. 2003. Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society of London Series B-Biological Sciences* 270, 2125-2132.

ICES, 1998. Report of the Baltic salmon and trout assessment working group. ICES Doc. CM 1998/ACFM: 17. ICES, Copenhagen.

ICES, 2003. Report of the Baltic salmon and trout assessment working group in Karlskrona, Sweden, 2–11 April 2003. ICES CM 2003/ACFM:20. 355.

Jarne, P. & Lagoda, P.J.L. 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution* 11, 424-429.
Kallio, I., 1986. Vaelluskalakantojen nykyinen tila ja hoito. Finnish Game and Fish Research Institute. *Monistettuja Julk.* 44, 1– 51.
Kangur, M. & Viilmann, M-L. 2001. Habitat, fish and fisheries. In: *Present and Potential Production of Salmon in Estonian Rivers* (eds. Wahlberg B, Kangur M), pp. 32–69. Estonian Academy Publishers, Tallinn.
Kazakov, R.V. & Titov, S.F. 1991. Geographical patterns in the population genetics of Atlantic salmon, *Salmo salar* L., on U.S.S.R. territory, as evidence for colonisation routes. *Journal of Fish Biology*, 39, 1–6.
Kauppi, R., Kvist, L., Ruokonen, M., Soivio, A. & Lumme, J. 1997. Lack of variation in mitochondrial DNA of the Baltic salmon (*Salmo salar*) indicates a bottleneck during or long before postglacial recolonisation of the Baltic Sea? *Oulanka Reports* 17, 19–23.
Kauer, M.O., Dieringer, D. & Schlötterer, C. 2003. A microsatellite variability screen for positive selection associated with the "out of Africa" habitat expansion of *Drosophila melanogaster*. *Genetics* 165, 1137–1148.
King, T.L., Kalinowski, S.T., Schill, W.B., Spidle, A.P. & Lubinski, B.A. 2001. Population structure of Atlantic salmon (*Salmo salar* L.): a range-wide perspective from microsatellite DNA variation. *Molecular Ecology* 10, 807-821.
Koljonen, M.-L., Jansson, H., Paaver, T., Vasin, O. & Koskiniemi, J. 1999. Phylogeographic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 1766-1780.
Koljonen, M.L., Tähtinen, J., Säisä, M. & Koskiniemi, J. 2002. Maintenance of genetic diversity of Atlantic salmon (*Salmo salar*) by captive breeding programmes and the geographic distribution of microsatellite variation. *Aquaculture* 212, 69-92.
Koskinen, M.T., Haugen, T.O. & Primmer, C.R. 2002. Contemporary fisherian life-history evolution in small salmonid populations. *Nature* 419, 826-830.
Laikre, L., Järvi, T., Johansson, L., Palm, S., Rubin, J-F., Glimsäter, C-E., Landergren, P. & Ryman, N. 2002. Spatial and temporal population structure of sea trout at the Island of Gotland, Sweden, delineated from mitochondrial DNA. *Journal of Fish Biology* 60, 49-71.
Landry, C., & Bernatchez, L. 2001. Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology* 10, 2525-2539.
Leonard, J.A., Wayne, R.K. & Cooper, A. 2000. Population genetics of Ice age brown bears. *Proceedings of the National Academy of Sciences of the United States of America* 97, 1651-1654.
Lewontin, R.C. 2002. Directions in evolutionary biology. *Annual Reviews in Genetics* 36, 1-18.
Li, Y.C., Korol, A.B., Fahima, T., Beiles, A. & Nevo, E. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Molecular Ecology* 11, 2453-2465.
Luikart, G. & England, P.R. 1999. Statistical analysis of microsatellite DNA data. *Trends in Ecology & Evolution* 14, 253-256.
Luikart, G., England, P.R., Tallmon, D., Jordon, S. & Taberlet, P. 2003. The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews. Genetics* 4, 981–994.
Manel, S., Schwartz, M.K., Luikart, G. & Taberlet, P. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* 18, 189-197.
Martin, S.A., Caplice, N.C., Davey, G.C. & Powell, R. 2002. EST-based identification of genes expressed in the liver of adult Atlantic salmon (*Salmo salar*). *Biochemical and Biophysical Research Communications* 293, 578-585.
Maynard Smith, J. & Haigh, J. 1974. The hitch–hiking effect of a favourable gene. *Genetical Research* 23, 23–35.
McConnell, S.K., O'Reilly, P., Hamilton, L., Wright, J.M. & Bentzen, P. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic

differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Sciences* 52, 1863-1872.
Moss, R., Piertney, S.B. & Palmer, S.C.F. 2003. The use and abuse of microsatellite DNA markers in conservation biology. *Wildlife Biology* 9, 243-250.
Nielsen, E.E., Hansen, M.M. & Loeschke, V. 1996. Genetic structure of European populations of *Salmo salar* L. (Atlantic salmon) inferred from mitochondrial DNA. *Heredity* 77, 351–358.
Nielsen, E.E., Hansen, M.M. & Loeschcke, V. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: A comparison of genetic composition over 60 years. *Molecular Ecology* 6, 487-492.
Nielsen, E.E., Hansen, M.M. & Mensberg, K-L.D. 1998. Improved primer sequences for the mitochondrial ND1, ND3/4 and ND5/6 segments in salmonid fishes: application to RFLP analysis of Atlantic salmon. *Journal of Fish Biology* 53, 216–220.
Nielsen, E.E., Hansen, M.M. & Loeschcke, V. 1999. Genetic variation in time and space: Microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution* 53, 261-268.
Nilsson, J. 1997. MtDNA and microsatellite variation in Baltic Atlantic salmon. *ICES Journal of Marine Science* 54, 1173-1176.
Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., Kohlmann, K., Löytynoja, A., Nielsen, E.E., Paaver, T., Primmer, C.R., Titov, S., Vasemägi, A., Veselov, A., Öst, T. & Lumme, J. 2001. Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonisation of the Baltic Sea area. *Molecular Ecology* 1, 89-102.
Norris, A.T., Bradley, D.G., Cunningham, E.P. 1999. Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* 180, 247-264.
Palva, T.K., Lehväslaiho, H. & Palva, E.T. 1989. Identification of anadromous and non-anadromous salmon stocks in Finland by mitochondrial DNA analysis. *Aquaculture* 81, 237–244.
Quinn, T.P. 1993. A review of homing and straying of wild and hatchery-produced salmon. *Fisheries Research* 18, 29-44.
Rexroad, C.E. & Palti, Y. 2003. Development of ninety–seven polymorphic microsatellite markers for rainbow trout. *Transactions of American Fisheries Society* 132:1214–1221.
Rise, M.L., von Schalburg, K.R., Brown, G.D. *et al.* (24 co-authors) 2004. Development and application of a salmonid EST database and cDNA microarray: data mining and interspecific hybridization characteristics. *Genome Research* 14, 478–490.
Rousset, F. & Raymond, M. 1997. Statistical analyses of population genetic data: new tools, old concepts. *Trends in Ecology & Evolution* 12, 313-317.
Saiki, R.K., Scharf, S. & Faloona, F. 1985. Enzymatic amplification of a-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230, 1350-1354.
Sanchez, J.A., Clabby, C., Ramos, D., Blanco, G., Flavin, F., Vazquez, E. & Powell, R. 1996. Protein and microsatellite single locus variability in *Salmo salar* L. (Atlantic salmon). *Heredity* 77, 423-432.
Schlötterer, C. 2002. A microsatellite-based multilocus screen for the identification of local selective sweeps. *Genetics* 160, 753-763.
Spidle, A.P. 2001. Fine-scale population structure in Atlantic salmon from Maine's Penobscot River drainage. *Conservation Genetics* 2, 11-24.
Stockwell, C.A., Hendry, A.P. & Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* 18, 94-101.
Sunnucks, P. 2000. Efficient genetic markers for population biology. *Trends in Ecology & Evolution* 15, 199-203.
Ståhl, G. 1987. Genetic population structure of Atlantic salmon. In: *Population Genetics and Fishery Management*. Edited by. N. Ryman and F. Utter. University of Washington Press, Seattle. pp. 121-141.
Säisä, M., Koljonen, M-L., Gross, R., Nilsson, J., Tähtinen, J., Koskiniemi, J. & Vasemägi, A. The genetic structure and postglacial colonization of Atlantic salmon into the Northern Europe on basis of DNA microsatellite data. *Submitted*.

Thorgaard, G.H., Bailey, G.S., Williams, D., *et al.* (2002) Status and opportunities for genomics research with rainbow trout. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology* 133, 609-646.

Tiira, K., Laurila, A., Peuhkuri, N., Piironen, J., Ranta, E. & Primmer, C.R. 2003. Aggressiveness is associated with genetic diversity in landlocked salmon, *Salmo salar*. *Molecular Ecology* 12, 2399-2407.

Tsoi, S.C.M., Ewart, K.V., Penny, S., Melville, K., Liebscher, R.S., Brown, L.L., & Douglas, S.E. 2004. Identification of Immune-Relevant Genes from Atlantic Salmon Using Suppression Subtractive Hybridization. *Marine Biotechnology* 6, 199-214.

Storz, J. F., Payseur, B.A. & Nachman,. M.W. 2004. Genome scans of DNA variability in humans reveal evidence for selective sweeps outside of Africa. *Molecular Biology and Evolution* 21, 1800-1811.

van Tienderen, P.H., de Haan, A.A., van der Linden, C.G. & Vosman, B. 2002. Biodiversity assessment using markers for ecologically important traits. *Trends in Ecology & Evolution* 17, 577-582.

Verspoor, E., & Jordan, W.C. 1989. Genetic variation at the *Me–2* locus in the Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology* 35A, 205–213.

Verspoor, E., McCarthy, E.M., Knox, D., Bourke, E.A., Cross, T.F. 1999. The phylogeography of European Atlantic salmon (*Salmo salar* L.) based on RFLP analysis of the ND1/16sRNA region of the mtDNA. *Biological Journal of Linnean Society* 68, 129-146.

Vigouroux, Y., M. McMullen, C. T. Hittinger, K. Houchins, L. Schulz, S. Kresovich, Y. Matsuoka, and J. Doebley. 2002. Identifying genes of agronomic importance in maize by screening microsatellites for evidence of selection during domestication. *Proceedings of National Academy of Sciences USA* 99, 9650–9655.

Vitalis, R., Dawson, K. & Boursot, P. 2001. Interpretation of variation across marker loci as evidence of selection. *Genetics* 158, 1811-1823.

Wang, J. & Whitlock, M.C. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163, 429-446.

Wang, D.Y.Y., Cannon, A. & Saunders, S.R. 2004. DNA species identification of archaeological salmon bone from the Pacific Northwest Coast of North America. *Journal of Archeological Science* 31, 619-631.

Waser, P.M. & Strobeck, C. 1998. Genetic signatures of interpopulation dispersal. *Trends in Ecology & Evolution* 13, 43-44.

Watson, J.D. & Crick, F.H.C. 1953. Molecular Structure of Nucleic Acids. *Nature* 171, 737-738.

Wennevik, V., Skaala, Ø., Titov, S.F., Studyonov, I. & Nævdal, G. 2004. Microsatellite variation in populations of Atlantic salmon from North Europe. *Environmental Biology of Fishes* 69, 143-152.

Wilding, C.S., Butlin, R.K. & Grahame, J. 2001. Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. *Journal of Evolutionary Biology* 14, 611–619.

WWF, 2001. The Status of Wild Atlantic Salmon: A River by River Assessment. World Wildlife Fond. http://www.wwf.org.uk/filelibrary/pdf/atlanticsalmon.pdf; pp. 1-184 (accessed 18-Aug-2004).

Yang, Z.H. & Bielawski, J.P. 2000. Statistical methods for detecting molecular adaptation. *Trends in Ecology & Evolution* 15, 496-503.

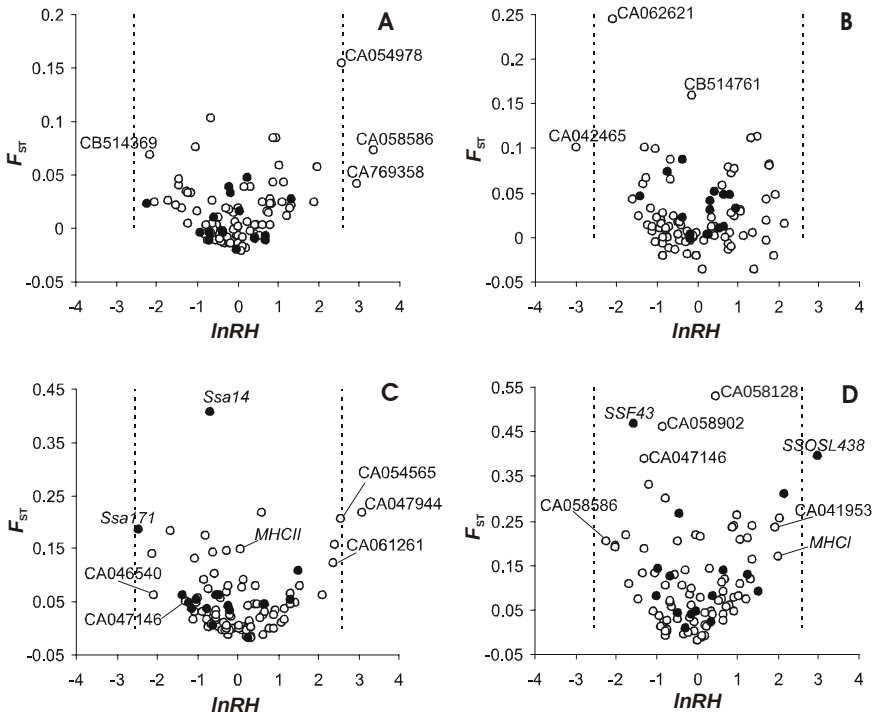

Figure 9. Plot of F_{ST} values against standardized ratio of gene diversity (*lnRH*) estimates for 79 EST associated (white bullets) and 17 genomic (black bullets) tandem repeat markers in small geographical scale. (A) R. Kitsa vs. R. Varzuga (B) R. Teno/Tana vs. R. Tuloma (C) R. Vindelälven vs. R. Torne/Tornionjoki (D) R. Taipale vs. R. Syskynjoki. Dashed lines indicate the 99% confidence interval (-2.58, +2.58) of standardized *lnRH* estimates. Accession numbers or locus names of putative candidate loci potentially affected by selection are shown close to the outlier or pointed by line.

Discussion and Conclusions

"*The best answer to any question about evolution is the lawyer's answer to any general question about the law: "It depends on the jurisdiction." That is why the program of evolutionary investigation never comes to an end—and, so often, never to a conclusion*".
Lewontin (2002)

Based on results of this thesis one can ask what are the most prominent evolutionary forces affecting the allele frequency distributions in Atlantic salmon populations? Clearly, there is no single universal answer. The study **I** revealed that the historical events, such as differentiation of salmon populations in isolated glacial refugia likely had significant effect on the present day distribution of genetic variability in Atlantic salmon. Study **II** demonstrated that surprisingly fast

Acknowledgements

I would like to express my sincere thanks to my supervisors for their steadfast support over these years. To Jan Nilsson, for his excellent guidance and for giving me the freedom to study anything I found interesting. Also, always finding time to discuss about various themes (including science) is highly appreciated. It is hard to imagine more thorough reviewer and better co-author than Riho Gross, whose work has undoubtedly improved the quality of the manuscripts. Lars-Ove Eriksson is thanked for his relentless support and interest of my work. Tiit Paaver has taught me a lot about aquatic biology from crayfishes to sturgeons. Also, your tireless efforts to teach me how to write 'proper' scientific text are very appreciated.

I want to thank all the co-authors for their contribution to the thesis in various stages. To Craig Primmer, for inviting me to PnP group and for the opportunity to do little more science than I might otherwise have done. To Mart Kangur, for cheerful field-trips on Estonian salmon and trout rivers and for sharing his broad knowledge in fish biology. To Marjatta Säisä, Marja-Liisa Koljonen, Jarmo Koskiniemi and Michael Hansen for internationalising my studies in Finland and Denmark. Also, I am indepted to many persons from several institutes and fish hatcheries for providing samples used in this thesis.

My warmest thanks to all the people in Vattenbruk, for good times both in- and outside of the Department. Without your help Ingela, our life in Sweden would be much more complicated. To Carin, for giving me an opportunity to teach fish genetics during the fish biology course over these years. To Bosse, who always knows how to fix everything (including computers, in no time) and Kjell, for generously adjusting my usually poorly-raised statistical issues. To Monica, Helena and Gersende for the company and help in the lab. To David and Johan S. for uncomplainingly sharing our overlapping 'work-territories'. To Henrik, for memorable fishing trips! To the former and present students (in broad sense) in Vattenbruk: Jens, Jeanette, Erik, Irina, Jason, Anna, Johan Ö., Rivi; PnP group: Heikki, Laura, J-P, Anni, Paula and basketball team 'Lilla Kolm'. Thanks!

My sincere thanks to my mother and father, my brother and his family for their support and encouragement. Aitäh!

To Katrin, for constant support, understanding and everything else... For Markus and Liisa, for being there and giving me totally different perspective of just everything.

This thesis was financed by the Royal Swedish Academy of Agriculture and Forestry (KSLA), Oscar and Lili Lamm's Foundation, Kempe Foundation, Estonian Science Foundation, the Centre of International Mobility (CIMO), the Finnish Ministry for Agriculture and Forestry, Nordic Academy for Advanced Study (NorFA) mobility scholarship, Danish Governmental scholarship, Spatial Ecology visitor's programme scholarship and Knut and Alice Wallenbergs scholarship.