Population divergence at small spatial scales

– theoretical and empirical investigations in perch

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Abstract
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Genetically structured populations arise when gene flow between groups of individuals is hindered by geographical, behavioural or temporal barriers. The identification of such groups is important for understanding evolution and has large implications for conservation concern. The field of population subdivision has received a lot of interest throughout the years and gained empirical support from a number of species. However, very little is known about population structure at small spatial scales, especially in a highly mobile species such as fish. The main object for my thesis was to further investigate population differentiation, explicitly at small spatial scales in the Eurasian perch. My results show that in this species, genetic differentiation occurs, even at very small spatial scales, both within lakes and in the Baltic Sea. Additionally, the differentiation can be stable over years and thus have a large impact in the evolution of adaptation to different environments. I also found barriers to gene flow that overlapped with the largest change in spring temperature, suggesting a temporal difference in spawning. Morphological differences were found at these small scales as well which indicates that a difference in food resources might be an underlying cause of change. My thesis work shows that the aquatic environment might not be as homogenous as widely thought and that there could be barriers or adaptations to different environments that hinder the fish from genetic panmixia. Slight patterns of isolation by distance (IBD) were found in the Baltic Sea, implying that the distance (i.e. currents) effect the level of differentiation via drifting of larvae and/or small fish. However, I have also theoretically investigated the IBD model of and seen that it is no longer correct when differences in population sizes are introduced. The pattern of IBD can mean high levels of gene flow or no gene flow at all, solely dependent on population size differences and fluctuations. My thesis has resulted in new and important findings regarding the existence and cause of genetic differentiation at very small spatial scales and thus added new knowledge into the field of evolution and speciation. In addition, my results also give insights into the contemporary state of the Eurasian perch and future evolutionary potential.

Keywords: Perca fluviatilis L., Perch, Small scale genetic differentiation, Morphometrics, Isolation by distance, Dispersal, Gene flow, Microsatellites, Spatiotemporal stability, Barriers

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List of Papers

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

I  S. Bergek, M. Björklund (2007). Cryptic barriers to dispersal within a lake allow genetic differentiation of the Eurasian perch. *Evolution*, 61: 2035-2041.

II S. Bergek, J. Olsson (2009). Spatiotemporal analysis shows stable genetic differentiation and barriers to dispersal in the Eurasian perch (*Perca fluviatilis* L.). *Evolutionary Ecology Research*, 11: 827-840.

III S. Bergek, M. Björklund (2009). Genetic and morphological divergence reveals local subdivision of perch (*Perca fluviatilis* L.). *Biological Journal of the Linnean Society*, 96: 746-758.

IV S. Bergek, G. Sundblad, M. Björklund. Population differentiation in Eurasian perch: Environmental effects on gene flow? *Submitted manuscript*

V Björklund M, Bergek S, Ranta, E, Kaitala V. The effect of local population dynamics on patterns of isolation by distance. *Submitted manuscript*

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Contents

Introduction..................................................................................................................7
  General background ...............................................................................................7
  Methods to study population differentiation ......................................................7
    Genetic markers ................................................................................................8
  Mechanisms .........................................................................................................8
    Biological ..........................................................................................................9
    Environmental .................................................................................................9
Population structure in fish ..................................................................................10
Study species ........................................................................................................11
Methods ................................................................................................................12
  Genetics .............................................................................................................12
  Morphometrics .................................................................................................12
  Simulations .........................................................................................................13
Aims of the thesis .................................................................................................13
  Main questions addressed; ..............................................................................13

Results and discussion ........................................................................................14
  Population differentiation possible within small lakes (paper I)? .................14
  Population structure temporally stable (paper II) ...........................................15
  Genetic divergence at small scales possible also in the Baltic Sea (paper III) .......................................................................16
  Environmental effects the level of gene flow (paper IV) .............................18
  The effects of local population dynamics on patterns of isolation by
distance (paper V) ..............................................................................................20

Conclusions and perspectives .............................................................................22

Sammanfattning på svenska ................................................................................24
  Bakgrund ..........................................................................................................24
  Syfte ...............................................................................................................25
  Resultat ...........................................................................................................25

Acknowledgements .............................................................................................27

References ............................................................................................................29
Introduction

General background
Genetically structured populations arise when gene flow between groups of individuals is hindered by geographical, behavioural or temporal barriers. The identification of such groups is important for understanding evolution as well as for conservation concern. Population structure is the first step towards reproductive isolation under several models of speciation and hence plays a central role in diversification and adaptation (Mayr 1963; Coyne & Orr 2004). Therefore, it is of great importance to fully understand population differentiation, on what spatial scales it can occur, what barriers to gene flow are acting and also how the environment might be affecting the pattern.

Interest in describing the genetic structure of a species has been within the field of population genetics for many years, beginning with Hardy (1908) and Weinberg (1908), who discovered the independent law of population genetics. This law predicts the outcome of genotypic and allelic frequencies in an ideal population and formed the basis of population genetic theories by S. Wright (1889-1988), R. Fisher (1890-1962) and J.B.S. Haldane (1892-1965). From 1931 and about fifteen years ahead, they produced many detailed results about the maintenance of genetic variation within populations. Complete knowledge about the genetics of a population would require description of the entire genome of each individual, which would be a very difficult and time consuming task. Therefore, during the last period of the 1900th century, technologies and models have been developed to allow investigation the population genetics and evolution of populations and species. The complexities of real populations are ignored and instead modelled by one or a few loci at one time in a population that is assumed to mate randomly, and if subdivided, follow simple migration models.

Methods to study population differentiation
A common tool to investigate and describe the genetic structure of populations are the $F$ statistics (i.e. $F_{ST}$) developed by Wright (1951) for his theory of adaptation in subdivided populations. The $F$ statistics are an important tool in measuring levels of inbreeding and the genetic distances between
populations and can thus lead to insights on population subdivision and divergence. $F_{ST}$ measures how much of the total heterozygosity ($H_T$) is explained by within population heterozygosity ($H_S$):

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

Genetic markers

There are a number of genetic markers that can be used to study the level of gene flow and genetic structure. Neutral ones, such as microsatellites or simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs) and amplified fragment length polymorphisms (AFLPs), have all been shown to be efficient in the analysis of phylogenetic relationships, population structure, gene flow and isolation by distance. In my thesis, I have however only used microsatellites.

Microsatellites consist of short non-coding DNA sequences that are repeated in tandem arrays and are species specific (Selkoe & Toonen 2006). The difference in number of repeats depends on mutation and hence, by looking at the variation of microsatellites in populations, inferences can be made about population structures and differences, genetic drift, genetic bottlenecks and even the date of a last common ancestor. They are easily amplified for identification by the polymerase chain reaction (PCR) process, using the unique sequences of flanking regions as primers. Another reason for using microsatellites is their assumed neutrality. This has however lately been challenged (see Nielsen et al. 2006), but the most common methods for estimating gene flow between populations ($F_{ST}$, rare alleles) have been shown to be relatively robust, at least to weak selection (Slatkin & Barton 1989).

Mechanisms

Members of a species rarely distribute themselves homogeneously in space; there is almost always some sort of clumping, shoaling, flocking, colony formation or other types of aggregation. It has for example been shown that migratory birds do not always disperse far from their natal site (Nagata 1993). In fact, the same observation has been seen in a number of animal and plant taxa (Rousset 1997 and references therein), which display site fidelity and as a result, show restricted dispersal.

Once a subdivision has occurred, the geographical structuring of genetic variation depends on the relative importance of natural selection, genetic drift, mutation and migration among populations. Genetic drift and mutations are stochastic events, leading to allele frequencies fluctuating in a random fashion and making it possible for populations to diverge. Both these
processes are much stronger in smaller populations. Selection affects population structure because different selection pressures likely exist in different populations, causing one trait in one population and another trait in another population to be favoured. However, since the genetic markers I have chosen to use in my thesis are supposed to be selectively neutral, selection should not play a direct role. Migration (gene flow) on the other hand is an important factor that homogenizes allele frequencies and leads to decreased genetic variation. There are a number of both biological and environmental mechanisms that can lead to population subdivision in the first place, either separately or in combination.

**Biological**

The ability to discriminate between kin and non-kin can have a large impact on the level of differentiation if the species aggregate according to kin. Kin-groups, where the populations aggregate according to kin and thus consist of relatives forming groups, can evolve by the presence of kin recognition or also without, just by the position of different families within different areas. Other biological mechanisms such as homing behaviour or site fidelity, can also lead to a nonrandom distribution of individuals with respect to their genotype and provide a mechanism by which genetic variation may be maintained (Sugg *et al.* 1996).

**Environmental**

Considering the environment, the geographical distance separating individuals is probably the factor that has received the most interest since it is easy to portray how gene flow decreases with increasing distance, at least when considering population structures at large spatial scales (Rousset 2004). This is incorporated in the model of Isolation by Distance (IBD) (e.g. Wright 1943). In this model, the level of gene flow (or number of migrants) is expected to decrease with increasing distance between populations under equilibrium conditions. Over time, limited migration produces genetic similarities among spatially close populations and larger differences between distant populations. The literature contains numerous examples of IBD from a wide variety of taxa including plants (Sharbel *et al.* 2000), freshwater and marine fishes (Planes *et al.* 1996; Hansen *et al.* 1998; Pogson *et al.* 2001), amphibians (Johansson *et al.* 2006; Rudh *et al.* 2007), birds (McDonald *et al.* 1999), and mammals (Pope *et al.* 2007). In addition to explaining genetic structure, IBD patterns can also help us to understand the interaction between migration and genetic drift (contemporary processes), and to link limited dispersal ability and genetic differentiation.

However, the physical ability to disperse can be a poor predictor of actual dispersal distributions (Rousset 1997) and there may be physical or other
barriers that may prevent migration among populations that are geographically close (Epperson 2003). Lately, a new field named landscape genetics, introduced by Manel et al. (2003), has been explored. This is because the environmental features of habitats can either restrict or promote movement of individuals in natural populations and consequently affect the extent of genetic connectivity. For instance, when the habitat is highly fragmented, genetic drift and mutation may lead to an overall patchy pattern of genetic variability (Rousset 2003). Over the past few years, many studies have investigated the field of landscape genetics and detected landscape and environmental features that affect the genetic structure of populations in, for example, the California mule deer (*Odocoileus hemionus*) (Pease et al. 2009), the common vole (*Microtus arvalis*) (Gaufre et al. 2008) and the wood frog (*Rana sylvatica*) (Lee-Yaw et al. 2009).

**Population structure in fish**

In fish and aquatic animals in general, investigating and understanding population structure is even more intriguing, since it is hard to follow dispersal and pin-point mechanisms behind barriers to gene flow and the development of genetic structure.

Fish are highly mobile species, capable of migrating over long distances. In addition, many species have a free dispersal larval phase, which should facilitate genetic panmixia over both small and larger geographical scales. Even though the potential for long distance migration and extensive gene flow exists, it has been shown in, for example, the coastal Atlantic cod (*Gadus morhua* L.), population structure among local populations on a scale that is considerably smaller than the dispersal ability of the species (Ruzzante et al. 2000; Knutsen et al. 2003). Water movement, i.e. currents, normally works as a homogenizing effect, but can also limit gene flow between populations, for instance, by retaining eggs and larvae in local current systems (Ruzzante et al. 1998; Stepien 1999; Nielsen et al. 2005). An accumulating amount of studies have also shown genetic differentiation (Palumbi et al. 1997; Bekkevold et al. 2005; Nielsen et al. 2005; Barluenga et al. 2006), despite the potential for widespread genetic panmixia. Most studies focus on larger geographical scales, but whether the same pattern is found at smaller spatial scales is less known (but see, Duffner et al. 2006; Narum et al. 2008; Thuesen et al. 2008; Purcell et al. 2009). Some studies have been able to link the differentiation to behavior, for example, to shoal with familiar conspecifics such as in guppies (Griffiths & Magurran 1999), three-spined stickleback (Barber & Ruxton 2000) and brown trout (Höjesjö et al. 1998). Behaviour such as natal homing has also been shown to lead to genetic structure within species (Thorrold et al. 2001). A spatial genetic difference could also
arise if populations are reproducing at different times within the reproductive season (McPherson et al. 2003; Jørgensen et al. 2005).

It is relatively easy to understand what environmental barriers restrict dispersal and gene flow on land, for example, via mountains, rivers or forests. Finding and distinguishing habitat discontinuities in aquatic environments is, on the other hand, more difficult. It has been shown, though, that environmental factors such as habitat barriers, including deep water, sandy areas and discontinuity, affect population structure in cichlids (Danley et al. 2000; Rico & Turner 2002). Additionally, salinity and temperature have been shown to affect and give rise to genetically distinct populations in, for example, herring in the Baltic Sea (Bekkevold et al. 2005; Jørgensen et al. 2005). Other landscape variables have also been shown in, for example, the Yellow perch (Leclerc et al. 2008), sticklebacks (McCairns et al. 2008) and Atlantic salmon (Dillane et al. 2008). So, with the last years, increased knowledge and technical methods make finding environmental barriers possible even in the aquatics. Geographical patterns on very small spatial scales, however, have rarely been explored.

Study species

The Eurasian perch (*Perca fluviatilis* L.) is a widely distributed fish in fresh- and brackish coastal waters throughout Europe. The females mature at an age between two and four years while males often mature one year earlier (Craig 2000). They spawn once a year in spring and the onset of it is temperature dependent and does not occur until the water reaches a temperature of ~7-8 degrees C° (Thorpe 1977; Saat & Veersalu 1996; Sandström et al. 1997). When the right temperature is reached, the female sheds her eggs in shallow water on vegetation or substrates in the littoral zone, which is followed by fertilization by nearby males (Thorpe 1977). After the eggs hatch, the larvae have a short pelagic phase (Wang & Eckmann 1994; Urho 1996) before metamorphosis and subsequent return to the littoral zone (Urho 1996). The perch has been shown to display a habitat- dependent divergence in morphology that is mainly due to phenotypic plasticity (Svanbäck & Eklöv 2002, 2003). Both habitat complexity and prey type diversity seem to influence morphological adaptations (Olsson & Eklöv 2005). Hence, the species is capable of morphological differences even within single lakes. There are mechanisms that speak both for and against finding also genetic differentiation in this species. Firstly, it has been shown in a previous study that adults are philopatric to their local site (Kipling & Le Cren 1984). Behrmann-Godel et al. (2006) also found that juvenile perch showed a preference for their own over an unfamiliar population, based on olfactory cues. This provides a mechanism for individuals to discriminate between populations which could also be used to avoid mating with other populations. Both
these behaviours should hence promote genetic structure. The pelagic nature of the larvae and mobility of adults suggest that the aquatic environment contains otherwise few physical barriers to dispersal, which would instead promote genetic panmixia.

Methods

Genetics
To investigate genetic differentiation in this species, I have used dinucleotide microsatellite loci developed for walleye (Wirth et al. 1999) and yellow perch (Leclerc et al. 2000). Fish were sampled by using gill nets in paper I, II and III and by small underwater detonations in paper IV.

Morphometrics
To investigate morphological differentiation (paper III), we used landmark based geometric morphometrics (Bookstein 1991). Geometric morphometrics is a collection of methods that deal directly with the coordinates of analogous landmarks, either in two or three dimensions, rather than with traditional distance or angle measurements. Hence, it allows fine-scale assessment of shape differences and, is therefore valuable for discerning patterns of intraspecific morphological variation and differentiation. These coordinate data are processed using Procrustes Superimposition, which translates, rotates and scales to unit size the specimen landmark configurations. Thus, size is separated from shape, enabling the separate analysis of these two components of morphology, as well as the analysis of their relationship. The procedure used was thin-plate spline analysis where a number of homogenous landmarks were digitalized on photos of fish (Fig. 1). I used the software TpsDIG32 (Rohlf 2003) to capture the x and y coordinates of each landmark. Superimposition of specimens and the calculation of both uniform and nonuniform shape components were done in TpsRelw (Rohlf 2003). To test whether the locations had an effect on shape, we performed a multivariate analysis of covariance (MANCOVA) on the partial warp scores. Although the effects of isometric growth are removed in the superimposition, the effects of allometry remain (Rohlf & Marcus 1993). Because of this, interaction of centroid size and location were tested. Patterns of among shape variation were examined by a Discriminant function analysis (DFA) of the partial-warp scores matrix, with the uniform component included. The DFA combines all scores obtained from TpsRelw into a few discriminant functions with a variable that maximally discriminates between locations. To visualise the morphological variation obtained by DFA, the individual discriminant variables for each function were imported into TpsRegr (Rohlf 2003) and regressed against the x and y coordinates of the original landmark positions.
Simulations
In paper V we used computerized simulations to investigate the model of isolation by distance further when relaxing the strong assumptions of the model.

Aims of the thesis
The main aims of my thesis were to investigate small scale genetic differentiation in a common fish species, to be able to understand the evolution of population structure, where can it happen, and why it happens.

Main questions addressed:

- Can there be population structure of perch in small lakes? (Paper I)
- Is the structure stable over time? (Paper II)
- Can there be population differentiation also in the Baltic Sea where the homogenizing effects of currents probably are stronger? Can there also be morphological differences at such small scales? (Paper III)
- Do the major barriers to gene flow correspond to changes in the environment? (Paper IV)
- What happens with the underlying genetic model used in IBD when relaxing the assumption of a constant effective population size? (Paper V)
Results and discussion

Population differentiation possible within small lakes (paper I)?

The aim of paper I was to investigate if barriers to dispersal and population differentiation are possible at all at small spatial scales in lakes.

Genetic divergence is often related to large geographical distances separating populations and restraining genetic exchange. However, very little is known about what patterns exists on small spatial scales and if barriers can be present even in apparently sympatric populations. Sympatric speciation, the formation of species without geographical isolation, is highly controversial. Despite strong theoretical objections, there are a number of studies demonstrating that sympatrically occurring species/populations are in fact sister-taxa. Such empirical evidence from nature for sympatric speciation is perhaps strongest among fishes where sister-taxa of stickleback, cichlid and salmon often reside in the same lake or river system (Schluter et al. 1992; Taylor et al. 1996; Taylor & McPhail 1999). Some of these are currently among the best recognised examples of sympatric speciation (Gislason et al. 1999; Schliwen et al. 2001; Barluenga et al. 2006). The primary reason why such cases are considered valid examples of sympatric speciation is the widespread argument that highly mobile fish are unlikely to face physical barriers to dispersal within their aquatic habitats (e.g. Coyne & Orr 2004). However, recent examples have indicated that cryptic barriers to dispersal may exist even in apparently homogenous aquatic environments.

To investigate genetic differentiation at small spatial scales, consistent with the existence of cryptic barriers to dispersal, I sampled perch from nine different locations in a small Swedish lake, Lake Erken, and used nine microsatellite markers.

The results revealed that a small but significant differentiation was present in the lake (global $F_{ST} = 0.023, P < 0.0001$). The result was not consistent with a traditional isolation by distance explanation or by comparing different kin-groups. Barriers to dispersal were, however, indeed present in the lake (Fig. 2). Although the physical features of the environment that caused the cryptic barriers to dispersal are currently unclear the major genetic discontinuity seemed to be situated in the centre and between littoral locations separated
by deep sections in the lake, indicating that open water might constitute one important barrier to the dispersal of perch.

Figure 2. Map of Lake Erken, sampled locations and depth. Lines represent areas of limited gene flow as identified by software BARRIER (Manni et. al. 2001). Number of lines represents the number of loci that supported the barrier.

The results show that perch within the lakes do not breed together and consist of one panmictic population. Genetic differentiation in highly mobile species such as fish can consequently exists, even on very small distances. Our results also suggest that findings of genetic divergence at small spatial scales from seemingly sympatric populations need to be analyzed in detail and in a continuous approach to exclude the possibility of cryptic barriers to dispersal (i.e., causing divergence in allopatry or parapatry).

Population structure temporally stable (paper II)
The aim of paper II was to investigate if the ‘cryptic barriers to dispersal’ are stable over time.

Stable structuring between adjacent populations might be expected to facilitate inter-population genetic and ecological differentiation and even speciation (Coyne & Orr 2004). Particularly in small isolated populations, drift and local adaptation will have important effects on gene frequencies and lead to increased divergence. Hence, understanding the stability of reduced gene flow and population structure is very important from both an evolutionary and conservational perspective. When genetic variation is investigated on a temporal scale, there are both biological and non-biological factors that can affect the outcome and give opposing results. Firstly, species with a larval phase might have very high fecundity but also high mortality in the early life stages (Hedgecock 1994). Hence, for a given year, most of the recruited young may be from very few parents because of a ‘sweepstake-chance
matching of reproductive activity’ (Hedgecock 1994). This would thus give both a high variance in progeny number and a very small effective population size, $N_e$, a phenomenon called the ‘Hedgecock effect’ (Waples 1998). This could then result in significant temporal variation of allele frequencies if different cohorts have been sampled. Hence investigating genetic differentiation over time is very important in order to avoid biased estimates.

To assess the temporal stability of the pattern, we sampled four of the same locations in Lake Erken used in the previous paper. The same approach was used with microsatellites to estimate the level of divergence.

We found the same pattern with a significant population structure ($F_{ST} = 0.025, P < 0.05$), low estimates of effective population size and low migration rates. Neither kin groups nor isolation by distance was the major reason for the differentiation this time either. Barriers present in the lake in 2004 (paper I) were located at the same positions two years later, indicating restricted gene flow over time.

The hierarchical analysis of molecular variance revealed that the proportion of the total genetic variance explained by differences between temporal samples within locations was of a similar magnitude as the proportion owing to spatial differences. Hence, there is a stable spatial structure at a certain geographical patch-size in the lake, but also a large temporal variation in allele frequencies at each location. This could be due to the presence of several small populations/schools, each belonging within a specific region (patch) of the lake and that different schools were sampled within each location at the two sampling events.

The results of this study reveal a stable spatial genetic differentiation in Lake Erken with barriers to dispersal located at the same place, at least over a sampling period of two years. Populations of perch appear to cluster in different patches in the lake that harbour genetically differentiated groups of fish. Hence, limited migration and barriers to dispersal can persist over time, even at a very small geographical scale and in an open aquatic environment.

Genetic divergence at small scales possible also in the Baltic Sea (paper III)

The aim of this paper was to investigate the possibility for small geographical scale population differentiation (genetic and morphological) in a ‘more open’ aquatic environment with an even more apparent lack of barriers to gene flow, the Baltic Sea. Secondly, we also wanted to address the role of distance in population structuring in this species explicitly within small areas.
Over time, limited migration produces genetic similarities among spatially close populations and larger differences between distant populations. The literature contains numerous examples of IBD from a wide variety of taxa. Identification of IBD patterns can help us to understand the interaction between migration and genetic drift (contemporary processes), and to link limited dispersal ability and genetic differentiation. In the coastal Atlantic cod (*Gadus morhua* L.), Ruzzante *et al.* (2000) and Knutsen *et al.* (2003) have shown population structure among local populations on a scale that is considerably smaller than the dispersal ability of the species. Thus, the discrepancies between the dispersal ability of the species and the actual movement of genes must be taken into account in studies of genetic differentiation and IBD.

To investigate the role of distance in the structuring of perch populations, we sampled fish at two different scales in the Baltic Sea. Seven locations and a total of 217 fish were sampled at the small scale and 203 individuals from six different locations from the large scale. Seven microsatellites were used to assess the level of genetic differentiation, and geometric morphometrics was used to investigate the level of shape differences.

Genetic differentiation was found on both spatial scales (global $F_{ST}$, $P < 0.05$). Very low estimates of migration rates between locations were detected at either scale, thus reinforcing the divergence process and making it possible for random genetic drift to act independently in each ‘population’. At the large scale, no effect of IBD was seen. At the small scale, however, the geographical distance had a slight effect on the level of genetic differentiation, suggesting very local dispersal ranges and gene flow. The MANCOVA analysis revealed significant morphological divergence, independent of size, between locations at the small scale ($P < 0.05$). The first two discriminant functions accounted for 73% of the total variation and hence were used in the regression analysis. The first DF was involved in a shape change along the y-axis, involving a change in body height. The second DF is characterized by shape changes along the x-axis, showing a slight bending of the body.

This study revealed genetic differentiation between locations on both a smaller (300 m- 2 km) and a larger (2 - 13 km) spatial scale, suggesting limitations to dispersal and gene flow. This is surprising given that the perch is a highly mobile species and in addition has a pelagic larval phase that is subject to drifting via currents over large distances. At the smallest scale, the distance had a slight effect on the differentiation. Morphological divergence between locations was also significant, congruent with a population subdivision at a microgeographic scale. Our empirical study shows the importance of taking small scale divergence into consideration when analysing populations for IBD, supporting theoretical studies, e.g. Epperson (2003).
Distance is usually seen as a major cause of genetic differentiation, but gene flow might be constrained for many reasons. Patterns at unpredictable places and geographical scales might be present, particularly in aquatic environments where barriers to dispersal are hard to discern.

Environmental effects the level of gene flow (paper IV)

The aim of this paper was to explore the ‘cryptic’ barriers to gene flow further to see if a correlation to the environment could be found.

On land, it is easy to understand how barriers to gene flow, for example via physical barriers such as mountains, rivers or forests can give rise to genetic divergence but understanding how it can arise in the aquatic environment is challenging. Understanding landscape effects on genetic connectivity provides insight into fundamental biological processes such as speciation and species’ distributions.

Environmental factors such as habitat barriers including deep water, sandy areas and discontinuity have been identified as barriers to gene flow in cichlids (Danley et al. 2000; Rico & Turner 2002), suggesting that intrinsic environmental heterogeneity may be sufficient to lead to local adaptation. How the environment shapes genetic divergence has been extended in ‘landscape genetics’, introduced by Manel et al. (2003). Relatively few studies have, however, been carried out testing the impact of the landscape on genetic structuring in fish populations, although changes in salinity and temperature have been shown to affect and give rise to genetically distinct populations in, for example, herring in the Baltic Sea (Jørgensen et al. 2005; but see also Bekkevold et al. 2005; Leclerc et al. 2008; McCairns et al. 2008). In addition, the existing literature has focused on genetic variation over large spatial scales while very little is known about how population structure is affected by environmental conditions on small spatial scales.

To investigate small scale genetic differentiation and the possible link to environmental parameters, we sampled fish along an approximately 30 km long archipelago gradient with diverse environmental conditions in the northern Baltic proper (Fig. 3). The inner parts are highly eutrophic and there is a general gradient with higher visibility and salinity towards the outer parts (Sandström & Karås 2002). Six microsatellite markers were used to detect the level of genetic differentiation. The environmental parameters were based on measurement at the sampling event and were represented by salinity, surface temperature, depth and turbidity. If mate choice is dependent on vision, turbid water might lead to a higher degree of gene flow between fish and thus homogenize the genetic variation of populations. Or, if fish actively avoid these waters, it might even constitute a barrier to gene flow. To test for any similarity between genetic difference between locations
and variation in the environments, a correlation analysis was done of PC1 and PC2 (based on allele frequencies in a principal component analysis conducted in PCAgen version 1.2 (Goudet 1999)) and the environmental parameters. In order to address and evaluate spatial barriers for gene flow related to temperature during spawning, remotely sensed spring temperatures were analysed using geographical information systems (ESRI ArcGIS and ERDAS Imagine).

Again, we found that the locations were slightly albeit significantly differentiated from each other \( (\text{global } F_{ST} = 0.013, P < 0.0001) \). Furthermore, pairwise comparisons showed that all but two locations (A and C) exhibited significant \( F_{ST} \)-values. A slight pattern of isolation by distance was found \( (P = 0.081) \). Principal component analysis revealed that PC1 and PC2 cumulatively explained 64% of the total genetic diversity. A correlation between PC1 and depth was found \( (P < 0.05) \), while no other environmental parameter was correlated with the genetic difference. The major break in genetic composition detected in the BARRIER analysis was situated between location A and B. This barrier matched and overlapped with the largest change in relative spring temperature, i.e. time of spawning (Fig. 3).

![Figure 3. Study area in the northern Baltic proper, sampling locations and the relative temperature difference in spring. Black solid lines represent how many loci supported the barrier to gene flow identified by the software BARREIR (Manni et al. 2004).](image)

In this study we showed genetic structure among perch populations as close as 3 km from each other along an archipelago gradient in the Baltic Sea. Interestingly, the biggest break in genetic variation overlapped extremely well with the steepest gradient in temperature difference during the time of spawning. Even though the temperature map was not collected the same year as the sampling event, it should reflect general temperature patterns in the archipelago during spring since the relative temperature difference between
the inner and outer parts of the archipelago is relatively stable between years. Selection of perch spawning habitat has been shown to be temperature dependent (Gillet & Dubois 1995) and there is evidence that perch show site fidelity (Willemsen 1977; Kipling & Le Cren 1984). This suggests that with some sites reaching the right temperature at different periods, a separation of time and space of spawning can indeed facilitate the divergence process, in agreement with Ruzzante et al. (2006). With time, this could potentially lead to perch becoming locally adapted to distinctive habitats, e.g. with different favourable spawning temperatures. Geographical distance did not seem to be the most important cause of structuring populations of perch at these short distances. However, because of the slight trend of IBD ($P$-value 0.081), the BARRIER analysis controlling for distance was rerun, with a genetic distance matrix consisting of residuals from the IBD analysis. But since the same pattern separating the inner from the outer archipelago was found, the barrier does not represent a decrease in gene flow because of the distance per se.

The results presented here strongly indicate that distance must not be the sole mechanism responsible for genetic differentiation, in congruence with studies on, for example, herring (Bekkevold et al., 2005; Jorgensen et al. 2005), and that the picture of population structure in the perch is complex with behavioural and environmental mechanisms acting together. To our knowledge, this is nonetheless the first study showing a correlation between genetic differentiation and environmental parameters within tens of kilometres, suggesting that environmental conditions can have a profound effect in interrupting gene flow also at surprisingly small spatial scales.

The effects of local population dynamics on patterns of isolation by distance (paper V)

The aim with this paper was to investigate the model of IBD and the robustness of the results when we allow for non-equal and fluctuating population sizes.

A feature of the theoretical models that predict an isolation by distance (IBD) relationship is the assumption of large population sizes, that are equal and constant over time (Wright 1943; Rousset, 2004). This is biologically a very strong assumption and most likely to be wrong in many cases (Whitlock & McCauley 1999). In nature populations vary in size, and are in many cases much smaller than assumed in the models. While it is clear that fluctuations in size have importance for the operation of drift and differentiation of a single population (Wright 1931, 1938), the effect on large-scale patterns, such as isolation-by-distance, of independent fluctuations in a set of
populations has not been explored. Biological populations are also strongly influenced by random variations in their environment. Random variations can be modelled by different environmental noise (white, red and blue, corresponding to random, positive and negative autocorrelation respectively). It has been shown that noise results in higher genetic differentiation among populations (Ranta et al. 2008). Hence, the effect of environmental variability is important at the genotypic level and could thus also affect the model and patterns of IBD.

To investigate this we analysed the effect on IBD when relaxing the assumption of large populations that are equal in size and constant over time, and additionally when incorporating environmental noise in the model.

We found in particular that if sampling has occurred along a population size-gradient, the probability of finding a significant pattern of IBD is substantially elevated, even if there is no gene flow at all between the populations. Hence, a pattern of IBD can mean high levels of gene flow or no gene flow at all, depending on how population sizes differ and how they fluctuate. Adding environmental noise gives even more unpredictable results where almost all simulated cases of noise gave a significant regression between geographical and genetic distances, even when no gene flow was allowed.

We found that when populations were stable over time and of equal size, the mean IBD was as expected, close to zero, and the probability of finding a significant pattern was close to the expected 5%. However, when a gradient in the population sizes was inferred, the pattern changed drastically and even though no gene flow was allowed, the mean IBD increased above zero, and the probability of finding a significant correlation was clearly higher than 5%. This should clearly not happen in a no gene flow scenario and evidently shows how different population sizes affect the pattern when analyzing for IBD. Fluctuations in population sizes affected the correlation as well, with a higher mean IBD pattern when population size fluctuated. Adding environmental noise gave similar results but gave a somewhat more unpredictable pattern.

This study shows that when the assumptions used in variable genetic analyses (large population sizes, that are equal and constant over time) are violated, the correlation with distance cannot be as easily inferred. In fact, they might even give opposite results.
Conclusions and perspectives

In this thesis, I address and investigate questions regarding genetic differentiation at small spatial scales in a common fish species, the Eurasian perch. This is important from a larger perspective since genetic differentiation is the starting point for reproductive isolation, i.e. speciation. Hence, understanding patterns on small spatial scales leads to a larger understanding of the evolution of species as well as of conservation concerns.

The field of population subdivision has gained a lot of interest throughout the years and has also been shown in a number of species. However, very little is known about the patterns at small spatial scales, especially in a highly mobile species such as fish. The main object for my thesis was to investigate population differentiation further, explicitly at small geographical scales, in what is thought to be a homogenous environment, the aquatics. I specifically also wanted to address mechanisms behind a potential pattern and investigate if genetic discontinuities correspond to differences in the environment. Especially now when the environment and climate are changing a lot, a deeper understanding of how this affects the evolution of population structure is even more important.

The results in this thesis clearly show that genetic differentiation can occur in the perch, even at very small scales both within lakes and in the Baltic Sea. The environment seems to play a role, where depth correlated to the biggest barrier in a lake (e.g. paper I) and also in the Baltic Sea (paper IV). In addition, spring temperature seems to have a large impact as well, since the biggest difference is found at the same place as the largest genetic difference (paper IV). This shows that the aquatic environment is not as homogenous as normally thought and that there could be barriers or adaptations to different habitats that hinder the fish from genetic panmixia. Since slight patterns of IBD were found (paper III and paper IV), the distance (i.e. currents) could have an effect, at least in the Baltic Sea where the wind is stronger and hence the dispersal of larvae or small fish is more affected. I have also found that even this kind of small geographical scale differentiation can be stable over years (paper II), suggesting it could have a large impact in the evolution of adaptation to different environments and maybe also different spawning habitats.

Even though the model and estimate of IBD can be useful, it makes a lot of assumptions which could give opposing results when violated (paper V).
I found that a pattern of IBD can mean high levels of gene flow or no gene flow at all, just depending on how population sizes differ and how they fluctuate. Hence, regressions between distance and genetic distance should be treated carefully and a more thorough knowledge of the population sizes and the ecology of the species are needed before drawing conclusions and making management actions.

To conclude from my thesis, it seems like more than one factor is probably acting for population differentiation to arise in the perch. An earlier study has shown a behaviour (kin discrimination) (Behrmann-Godel et al. 2006) that could be a mechanism for how they stay in subdivided populations, allowing genetic differentiation to evolve. And as shown in my thesis, also variation in the environment, mostly depth and temperature, seems to affect and explain, at least to some degree, a spatial pattern. I also found that morphological divergence can occur as well, even at these small scales and in the same habitat (littoral), suggesting a difference in food resources might be present and be an underlying cause of change. This has important implications since the ability for a species to be plastic and respond to different environmental variations may play an important role and facilitate genetic evolution.

Questions never end, and even though I have been able to answer some in my thesis, some still exist. To further test the effect of environment in the divergence process of perch, a larger survey should be conducted, investigating the same environmental variables in many areas. In addition, to be sure that the differentiation and patterns of IBD do not reflect a difference in effective population size, more temporal estimates should be used to give a more precise estimate of $N_e$. Another interesting question that my thesis has led to is to investigate the spawning habitats a bit further. It seems like there might be different spawning areas and/or a temporal difference in the environment (through temperature) when it occurs. Investigating spawning areas and collecting larvae should add knowledge about the population structure at a very early stage in life. If that reflects differences in the same directions as the older fish I have used in my studies, we can be even more certain the groups of fish in each location are well defined populations that stay together in the same aggregations/schools throughout the whole life.

To fully understand ecology and speciation, a detailed knowledge is needed about patterns of genetic differentiation within species. How can it arise, at what spatial scales and what mechanisms are affecting the pattern? This thesis has lead to some new and important findings regarding the existence of genetic differentiation at very small spatial scales. This has hopefully also added new knowledge into the field of evolution and speciation and in addition, also given insights on the contemporary state and future evolutionary potential of the Eurasian perch.
Sammanfattning på svenska

Bakgrund

Genetiskt strukturerade populationer uppkommer då genflöden mellan grupper hindras av geografiska, beteendemässiga eller temporala barriärer. Att identifiera sådana grupper är mycket viktigt både ur ett evolutionärt och bevarandemässigt perspektiv. En uppdelning på populationsnivå är det första steget mot reproductiv isolering och är sålunda en viktig del i artbildningens utveckling. Därför är det mycket viktigt att förstå hur populationer kan delas upp, vilka geografiska skalar det kan ske på och vilka ”barriärer” som finns för genflöden. Det är även viktigt att undersöka hur miljön kan påverka mönstret då det under detta årtionde sker stora förändringar i det avseendet.

Att en genetisk skillnad kan byggas upp mellan populationer är sedan länge känt hos många arter. Om detta även kan ske på korta avstånd är dock mindre undersökt. Framförallt i den akvatiska miljön, där genflöden mellan olika ställen torde vara stor på grund av den homogeniserande effekten att driva med strömmar och avsaknad av fysiska barriärer för spridning.

Det finns flera mekanismer som påverkar om en genetisk struktur kan uppkomma, både beteendemässiga och miljömässiga. Beteendemässiga mekanismer är exempelvis då individer som är släkt tenderar att gruppera sig med varandra. Ett annat beteende som påverkar populationsuppdeleningen är när individer är efter år återkommer till samma ställe för att reproduera sig. Även att populationer reproducerar sig vid olika tider på året, beroende av miljön (t.ex. temperaturen), kan leda till en genetisk skillnad mellan olika områden. Det har länge ansetts att den största miljömässiga mekanismen bakom en genetisk uppdelden är det geografiska avståndet som separerar populationer. Det betyder att populationer långt ifrån varandra är mindre genetiskt lika än närliggande. Detta är emellertid beroende av hur arter sprider sig och vilka hinder för spridning som finns. De senaste åren har det lagts mer fokus på miljön och det område djuren befinner sig i. Miljön behöver inte vara så homogen som man kan tro och det har visat sig att landskapet i sig kan innehålla barriärer för spridning och genflöde. På land är det lättare att förstå hur exempelvis vägar, floder, hav, berg och sjöar kan påverka djurs spridning och därmed ge upphov till genetiskt skilda populationer. Däremot är det svårare att förstå vad som ger upphov till barriärer i den akvatiska miljön. Uppenbara fysiska hinder som öar och vattenfall påverkar
självklart spridningen av organismer och därmed den genetiska strukturen. Det har även under de senare åren visats att det finns egenskaper i själva vattnet, exempelvis temperatur och salthalt, som kan leda till att populationer på olika ställen är anpassade till olika miljöer och därmed skapar en ”barriär för genflöde” och upphov till en populationsstruktur.

Abborre (*Perca fluviatilis* L.)
Abborren är en mycket vanlig fiskart som finns i både söt- och bräckt vatten i Sverige och övriga Europa. Den har visat ha beteenden som skulle kunna leda till en genetisk struktur, bl.a. genom att återkomma till specifika lekhabitat år efter år. Det har även visats att de genom lukt kan känna skillnad på populationer och väljer att simma mot de populationer som de själva stammar från. Detta är två mekanismer som skulle kunna bidra till en genetisk uppdelning av populationer. Däremot har abborren även en pelagisk larvfas på ca 3-4 veckor där de driver runt i pelagiskt vatten, enbart påverkad av strömmar och vind. Detta är något som i stället skulle kunna leda till homogeniserande av den genetiska variationen, bara genom en slumpartad fördelning av larver i vattnet. Det finns alltså mekanismer som talar både för och emot en genetisk struktur hos arten.

**Syfte**
Syftet med min avhandling var att undersöka om en genetisk struktur är möjlig hos abborren. Detta ville jag studera både i sjöar och i öppnare system som Östersjön. Vad gäller Östersjön är det möjligt att strömmar och vind har en större påverkan och därmed kan motverka en genetisk struktur. Mina studier genomfördes på något större avstånd (< 30 km) men även på extremt korta avstånd (300 m). Detta skulle kunna ge mer information om vilka geografiska skalar en genetisk skillnad kan uppstå. Jag undersökte även om en eventuell populationsstruktur kan vara stabil över tid och vad som kan tänkas påverka och ge upphov till ett genetiskt mönster.

**Resultat**
Mina resultat visar tydligt att en genetisk uppdelning av abborrpopulationer kan förekomma trots mycket korta avstånd, både inom sjöar och i innanhav som Östersjön. Miljön verkar ha viss betydelse i denna uppdelning då den största genetiska skillnaden verkade stämma överens med djupa delar i sjön (uppsats I) och även i Östersjön (uppsats IV). Värtemperaturen verkar spela ytterligare en roll, vilket indikerades i uppsats IV där den största genetiska skillnaden inföll på samma ställe där skillnaden i temperaturen var störst. Detta tyder på att den akvatiska miljön inte är så homogen som man kan tro
och att det kan finnas ’barriärer’ i vattnet som inte bara är fysiska (t.ex. öar eller dammar m.m) som påverkar uppkomsten av en genetisk struktur hos abborren. Jag fann även en viss korrelation mellan den genetiska skillnaden och det geografiska avståndet (IBD) mellan populationer (uppsats III och IV) i Östersjön vilket kan tyda på att strömmar har en större effekt på ynglens ålder i larvstadiet i dessa miljöer. Vinden kan tänkas ha en större påverkan i Östersjön än i slutna sjöer eftersom det finns större områden med ’öppet vatten’ där larverna kan driva längre sträckor. Resultaten visar även att denna typ av genetisk uppdelning kan vara stabil över tid (uppsats II) vilket kan ha en stor påverkan ur ett evolutionärt perspektiv.

Eftersom jag har undersökt avståndets inverkan på den genetiska strukturen och sett både samband men även att populationer kan vara väldigt avvikande från varandra trots att de är närliggande, ville jag även undersöka hur modellen för sambandet mellan det geografiska avståndet och den genetiska skillnaden fungerar och vad som händer om det finns lokala variationer som gör att kraven för modellen bryts (i.e. populationsstorlek och fluktuationer i miljön). Det visade sig att modellen inte fungerar så väl när undersökta populationer har olika och fluktuerande storlekar. Signifikanta mönster för en korrelation mellan genetisk och geografisk struktur kunde hittas, trots att inget genflöde var tillåtet. Detta betyder att modellen för IBD inte är helt robust och det verkade som om framförallt en gradient i storleken på populationerna (antal individer) påverkade modellen mest. Detta betyder att resultat från traditionella IBD-analyser borde ses med lite mer skepsis och även inkludera information om populationsstorlekar för att en ordentlig granskning av avståndets påverkan på den genetiska strukturen ska kunna genomföras.

Sammanfattningsvis visar jag i min avhandling att en genetisk struktur kan uppkomma även hos arter som lever på mycket korta avstånd i homogena miljöer, som den akvatiska miljön och att denna struktur även kan vara stabil över tid. Miljön kan innehålla ’kryptiska barriärer’ för spridning/genflöden vilket gör att en genetisk uppdelning kan uppkomma. Det är således mycket viktigt att undersöka alla aspekter och barriärer eftersom genflöden inte behöver vara så enkla som direkta fysiska hinder.
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