Genetic variation and inference of demographic histories in non-model species

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Abstract

Both long-term environmental changes such as those driven by the glacial cycles and more recent anthropogenic impacts have had major effects on the past demography in wild organisms. Within species, these changes are reflected in the amount and distribution of neutral genetic variation. In this thesis, mitochondrial and microsatellite DNA was analysed to investigate how environmental and anthropogenic factors at different spatial and temporal scales have affected genetic diversity and structure in four ecologically different animal species.

The glacial cycles are considered to have played an important role in the history and distribution of species. Paper I describes the post-glacial recolonisation history of the speckled-wood butterfly (Pararge aegeria) in Northern Europe. A decrease in genetic diversity with latitude and a marked population structure were uncovered, consistent with a hypothesis of repeated founder events during the postglacial recolonisation. Moreover, Approximate Bayesian Computation analyses indicate that the univoltine populations in Scandinavia and Finland originate from recolonisations along two routes, one on each side of the Baltic.

Paper II aimed to investigate how past sea-level rises affected the population history of the convict surgeonfish (Acanthurus triostegus) in the Indo-Pacific. Assessment of the species’ demographic history suggested a population expansion that occurred approximately at the end of the last glaciation. Moreover, the results demonstrated an overall lack of phylogeographic structure, probably due to the high dispersal rates associated with the species’ pelagic larval stage. Populations at the species’ eastern range margin were significantly differentiated from other populations, which likely is a consequence of their geographic isolation.

In Paper III, we assessed the effect of human impact on the genetic variation of European moose (Alces alces) in Sweden. Genetic analyses revealed a spatial structure with two genetic clusters, one in northern and one in southern Sweden, which were separated by a narrow transition zone. Moreover, demographic inference suggested a recent population bottleneck. The inferred timing of this bottleneck coincided with a known reduction in population size in the 19th and early 20th century due to high hunting pressure.

In Paper IV, we examined the effect of an indirect but well-described human impact, via environmental toxic chemicals (PCBs), on the genetic variation of Eurasian otters (Lutra lutra) in Sweden. Genetic clustering assignment revealed differentiation between otters in northern and southern Sweden, but also in the Stockholm region. ABC analyses indicated a decrease in effective population size in both northern and southern Sweden. Moreover, comparative analyses of historical and contemporary samples demonstrated a more severe decline in genetic diversity in southern Sweden compared to northern Sweden, in agreement with the levels of PCBs found in the respective areas.
List of papers

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


* These authors contributed equally to the study.
† These authors contributed equally to the senior position.

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“Wonder is the beginning of wisdom”
Socrates 470 BC – 399 BC
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Introduction

“Nothing in biology makes sense except in the light of evolution.”

Theodosius Grygorovych Dobzhansky - 1973

The amount and distribution of neutral genetic variation in extant organisms is a consequence of their history. At a large time scale, historical climate-driven effects such as glacial cycles (Paper I) and sea-level changes (Paper II) have played a major role in influencing genetic diversity. At shorter time scales, human activities such as overexploitation of resources (Paper III), pollution (Paper IV), habitat destruction and introduction of exotic species have had major impacts on biodiversity. The influence of these processes on the history of populations and their genetic diversity can be assessed using genetic tools.

Genetic diversity and population genetics: the basis to infer population histories

The inference of demographic parameters from genetic data is based on the fact that evolutionary forces change the frequency of alleles in a population through time. The process of mutation creates new alleles and increases genetic variation. Mutations can be neutral, or have positive as well as negative effects on the fitness of an individual. The frequency of an allele in a population can thus increase or decrease under the effect of natural selection (Darwin 1859; Fisher 1930), depending on whether its beneficial or deleterious to reproductive success. The frequency of an allele may also change due to random sampling of alleles from one generation to the next, called genetic drift. The rate of genetic drift in a population is directly dependent on the effective population size (Wright 1931). In a population with small effective population size, genetic drift is more pronounced and can lead to the fixation or loss of alleles. However, in a large enough population without migration or selection the effect of genetic drift can be negligible. In such situations, mutation-drift equilibrium can be maintained, where the loss of diversity through genetic drift is compensated by the introduction of diversity through mutation. In addition, migration, or in evolutionary terms, the movement of alleles between populations (gene-flow) will tend to homogenize allele frequencies between populations, in absence of selection. Gene flow among populations can take place via the dispersal of animal organisms, planktonic larvae, seeds or even gametes.

However, in natural situations, individuals within a species rarely breed randomly (under panmixia). This non random-mating can be due to species-specific life-history traits such as philopatry, ecological factors such as habitat preferences, or environmental barriers such as mountains or rivers. As a consequence, within a geographic range, individuals are typically more closely related to each other com-
pared to individuals from different geographic regions, creating genetic differentiation among groups of individuals. This type of genetic structure within species leads to the formation of genetically distinct populations.

Knowledge on how these forces drive changes in the frequency of alleles, and depending on the spatial and temporal scale of interest, different patterns of genetic differentiation and population history can be identified. For example, population genetic models can be used to reconstruct the demographic history of a population in terms of expansions and reductions in effective population size as well as population divergence. Since also the timing of such events can be estimated, it is also possible to explore the interaction between past changes in demography and historical geological or climatic conditions.

**Reconstructing past demography**

Since the advent of DNA sequencing in the early 1990s and the development of the fields of human evolution, phylogeography and conservation biology, the interest of estimating demographic parameters and reconstructing the past demography of populations increased (Avise 2000; Beaumont 2004; Emerson et al. 2001). This investigation was facilitated by the development and application of the coalescence theory (Hudson 1991; Kingman 1982a; Kingman 1982b).

**The coalescent theory**

The coalescent theory is a population model looking back in time to interpret genetic data. Thus, the coalescent describes the genealogical history of a sample of individuals from a population back until their Most Recent Common Ancestor (MRCA). Using the coalescent theory, it is possible to estimate population parameters, such as effective population size, and to investigate possible population size changes. Population size changes can be reflected in the shape of the genealogy of the coalescent. For example, a population under expansion produces long external branches in the genealogy, resulting in an excess of singletons. A class of statistical tests (such as Tajima’s $D$ test), using the frequency of segregating sites, has been developed and measure whether there is an excess or a deficiency of rare mutations in the observed dataset compared to expectation under the Wright-Fisher model (Fu & Li 1993; Tajima 1989a, b). The results are often interpreted in terms of population size changes.

**Identification of population expansions and bottlenecks**

Historically, to detect population expansion, analysis of the distribution of pairwise differences among DNA sequences, also called mismatch distributions, has been used (Excoffier & Schneider 1999; Harpending 1994; Rogers & Harpending 1992; Sherry et al. 1994; Slatkin & Hudson 1991). Following a sudden population size expansion, a population displays a unimodal distribution of pairwise differences. The timing of the start of population growth can then be estimated by the position of the peak in the distribution (Rogers & Harpending 1992). Other statistical tests, such as Fu’s $F_s$, based on haplotype distributions have also been developed (Fu 1997).
In conservation biology, detecting population bottlenecks is essential since declines in genetic diversity of a population may have a negative effect on its ability to survive. Population bottlenecks can leave distinctive signatures in expected heterozygosities and in the distributions of allele sizes. Genetic bottleneck tests typically make use of these properties to determine if a population has gone through a demographic decline. The earliest population bottleneck tests aimed at detecting departures from expectation under mutation-drift equilibrium. For example, tests such as “heterozygosity-excess” (Cornuet & Luikart 1996; Piry et al. 1999) and the M-ratio test (Garza & Williamson 2001) have been extensively used (Peery et al. 2012). Nonetheless, the statistical power to detect genetic bottlenecks using these approaches appears to be limited, especially if the bottleneck occurred only a few generations before sampling (Aguilar et al. 2008; Girod et al. 2011; Hoffman et al. 2011; Peery et al. 2012). However, more advanced methods to estimate demographic parameters and characterize demographic histories are rapidly being developed (Beaumont 2004, 2010).

**Inferring more complex demographies: new tools, new possibilities**

Recently, more powerful approaches based on maximum likelihood, Bayesian and Approximate Bayesian Computations (ABC) have been developed (Bertorelle et al. 2010; Kuhner 2009). For example, Bayesian skyline plots can provide estimates of changes in effective population size through time (Drummond & Rambaut 2007; Drummond et al. 2005; Ho & Shapiro 2011; Pybus et al. 2000). Bayesian methods implemented in MSvar show a higher probability of detecting population bottlenecks compared to more traditional heterozygosity-excess and M-ratio tests (Girod et al. 2011; Storz & Beaumont 2002). In addition, packages such as BEAST (Drummond & Rambaut 2007), LAMARC (Beerli & Felsenstein 2001; Kuhner et al. 1998) and SPLATCHE2 (Ray et al. 2010) also allow estimation of effective population sizes under different population histories.

The ABC-framework allows comparison of different scenarios of evolution, in order to select the best scenario and to estimate posterior distributions of model parameters. In brief, prior distributions of parameters describing various aspects of the scenarios are given by the user. Following this, a high number of simulations with parameter values drawn from the prior distributions are performed. Summary statistics are then computed for the observed dataset, as well as for each simulated dataset. The simulations with summary statistics that are most similar to those of the observed data are kept. This allows for model selection to be implemented, as well as recovery of posterior distributions of parameters from the selected model. Model checking and cross validation can also be performed using pseudo-observed datasets (Bertorelle et al. 2010; Csillery et al. 2010).
Consequences of historical changes in the environment

Describing the history of populations and the past variation in population size is essential to understand the impact of past climate changes on the current distribution of species, but also for the conservation of endangered species.

Glacial-cycles - speckled wood butterfly
During the Quaternary (2.6 Myr ago to today), fluctuations in the Earth’s climate have led to several glacial episodes, and these have played an important role in the abundance and distributions of species. For instance, during the Last Glacial Maximum (LGM, around 23,000-18,000 years ago), many temperate species in the northern hemisphere were confined to southern refugia (Hewitt 2000; Hewitt 1999; Taberlet et al. 1998) while, in contrast, arctic species had a much larger distribution than they do today (Stewart et al. 2010). After the LGM, during the Pleistocene-Holocene transition, the ice-sheets covering much of northern Europe and North America melted. Accordingly, some species adapted to cold conditions became constrained to smaller geographical areas, and depending on their adaptations and environmental tolerance, decreased in population size and genetic diversity (Campos et al. 2010) or even went extinct (Stuart & Lister 2011). In contrast to cold-adapted species, temperate species were able to recolonise high-latitude regions and consequently expanded in population size. Such postglacial recolonisation processes left genetic footprints both in terms of genetic diversity (Lessa et al. 2003) and genetic relatedness among populations (Hewitt 1999).

Insects and particularly butterflies are good models to study evolutionary processes and climate-driven range shifts (Hill et al. 2011; Hill et al. 1999; Parmesan et al. 1999). The speckled wood butterfly, Pararge aegeria, was likely confined to southern refugia during the LGM and subsequently expanded northwards. Today the species is found in Scandinavia and Finland, which constitute its northern range margin. However, little is known about the recolonisation history of the species, nor its genetic diversity and structure in Northern Europe.

Sea-level fluctuations - convict surgeonfish
The Indian and Pacific oceans contain the highest concentration of tropical marine biodiversity (Ekman 1953) and are divided into several biogeographical provinces (Briggs & Bowen 2012; Cowman & Bellwood 2013; Kulbicki et al. 2013). During the LGM, the sea-level was about 120 meters lower than it is at present-day, which led to a considerable reduction in connectivity between the Indian and Pacific oceans (Sathiamurthy & Voris 2006; Voris 2000). In addition, the distribution and abundance of coral reefs were much lower than those of today (Kleypas 1997; Ludt & Rocha 2014). By inferring past events in coral reef taxa, one could expect to find patterns of ancient vicariance during the last ice age, demographic expansion at the end of this period, and Holocene high levels of gene flow.

One of the most abundant and widespread coral reef fish in the Indian and Pacific oceans is the convict surgeonfish, Acanthurus triostegus. Its range spans several biogeoetric regions, from East Africa through the Indo-Pacific to the eastern
Pacific. Compared to other widespread fish, in which little genetic structure has been found based on mitochondrial DNA (Horne et al. 2008; Klanten et al. 2007), high levels of differentiation have been described in Acanthurus triostegus using allozymes (Planes & Fauvelot 2002). Therefore, we were interested in studying the population structure using mitochondrial DNA, and infer the demographic history of the convict surgeonfish.

Recent anthropogenic impacts

From hunter-gatherer societies, through farming and pastoralist societies, to modern-day societies, humans have exploited and modified their environment to meet their needs. However, with the progress of technology, especially following the industrial revolution, pollution as well as overexploitation of natural resources (through fishing, hunting, agriculture, animal farming and forestry) have become more intense and today constitute major threats to biodiversity.

Harvesting pressure – moose in Sweden

Many species have become extinct due to human overharvesting, both in the terrestrial and marine environments, such as the dodo, the Tasmanian tiger, the great auk and Steller’s sea cow. Others species, such as most whales and many shark species have decreased dramatically in population size (Baum et al. 2003; Diamond 1989; Jackson et al. 2001; Roberts & Solow 2003). Harvesting can reduce the effective population size down to critical levels where genetic drift and inbreeding can become a threat to the survival of the population. The genetic diversity of a population and its spatial structure can also be modified due to intensive harvesting. Moreover, selective harvest can change the genetic composition of a population (Allendorf et al. 2008).

In order to determine which populations to manage and protect, it is important to define conservation and management units. Thus, concepts such as “Evolutionary Significant Units” and “Management Units” have been discussed and are based mainly on genetic parameters (Crandall et al. 2000; Moritz 1994; Palsboll et al. 2007; Wailes & Gaggiotti 2006). These aspects have been well studied in many taxa, but several studies have also underlined the need to estimate and delineate population genetic structure and to take into consideration the demographic history of the population (Manel et al. 2004; Taberlet et al. 1995; Tallmon et al. 2004; Waits et al. 2000). Thus, population genetic data can provide valuable information to monitor populations and species for management and conservation (Schwartz et al. 2007).

The largest game animal in Sweden is the moose, Alces alces. Approximately one third (c. 100,000 animals) of the population is currently harvested annually. However, in the beginning of the 19th century a strong decline in population size occurred. It is believed that this decline culminated with only a few hundred to a few thousand animals remaining in the central part of Sweden. The population size has subsequently increased rapidly since the 1960s (Lavsund et al. 2003). Although the
current demography of the population is well studied, little is known about its population structure and past demographic history, despite the importance of these parameters for the management of the Swedish moose.

**Environmental toxins – Eurasian otters in Sweden**

Some of the most common toxins found in the environment are polychlorinated biphenyls (PCBs), pesticides, phthalates and heavy metals. Depending on their concentration in organisms, these compounds can be highly toxic and have tremendous effects on a wide range of organisms. They can lead to increased mortality, reduced fertility and/or reduced reproductive rates. Thus, these toxins can result in major decreases in population size, and can reduce the genetic diversity of populations. For example, DDT, an organochlorine used as insecticide in the 1940s and banned in most countries since the 1970s, poisoned the wildlife for decades due to its hydrophobic and lipophilic properties and its high bioaccumulation potential. According to a well-described mechanism, a metabolite of DDT, called DDE, caused eggshell thinning that led to egg breakage and death of embryos. This resulted in severe population declines in bird species in both North America and Europe (Bignert et al. 1995; Bowerman et al. 1995; Green 1998).

Another class of contaminants with high impact on biodiversity is PCBs. In the Baltic ecosystem and in Sweden, both PCBs and DTT have impacted the environment and the fauna (Olsson & Reutergårdh 1986). For example, studies have shown a negative impact on the Baltic guillemot (Bignert et al. 1995; Jorundsdottir et al. 2006), the white-tailed sea eagle (Hailer et al. 2006; Helander et al. 2002), seals (Bredhult et al. 2008; Nyman et al. 2003) and Eurasian otters (Olsson & Sandegren 1991; Roos et al. 2001). The latter species, *Lutra lutra*, was common in Sweden before the 1950s but went through a drastic bottleneck between the 1950s and 1980s. After the bans of DDT and PCBs in the 1970s, the population began to recover. However, the genetic consequences of this demographic bottleneck have remained unknown, both in terms of how much genetic variation was lost and whether the bottleneck had an effect on present-day population structure.
Objectives

The aim of this thesis was to reconstruct past demography and assess population structure by estimating genetic variation in four wild animal species living in different environments.

More specifically, the objectives were to:

- Assess species demographic histories using inferences based on deviations from mutation-drift equilibrium as well as coalescent-based approximate Bayesian computation (Papers I – IV).

- Evaluate the relationship between inferred demographic changes and past climatic (Papers I & II) as well as anthropogenic (Papers III & IV) changes.

- Examine to what extent genetic structure among contemporary populations have been affected by past changes in climate (Papers I, II & III) and human-mediated bottlenecks (Papers III & IV).

- Investigate the relative amount of genetic diversity and population differentiation at species range margins (Papers I & II).
Materials and methods

Several species that inhabit widely different environments were used in this research, and a range of analyses using genetic information were conducted to learn both about the population history of each species and understand the factors influencing its distribution. The taxonomic as well as environmental diversity in these studies comprised terrestrial invertebrates and mammals (Papers I and III), a semi-aquatic mammal (Paper IV) and a marine fish (Paper II). Both recently collected samples (Papers I – IV) and historical museum samples (Paper IV) were analysed to examine the population structure and demographic history and each respective species.

Laboratory methods

Samples
In Paper I, speckled wood butterflies (n=209) were collected between 1984 and 2011 from locations in northern Europe, but with particular emphasis on Sweden. In Paper II, convict surgeonfishes (n=179) were collected between 1994 and 2008 from reef slopes or lagoons at several sites across the Indo-Pacific. In Paper III, a very large number of fresh tissue samples (n=20,358) were obtained from moose killed throughout Sweden during one single hunting season in 1980. A smaller number of these moose samples were genotyped for microsatellite and mitochondrial DNA variation (n=1207 and n=48, respectively). In Paper IV, European otters (n=139) were sampled at three time points, before 1950 (n=17), between 1950 and 1979 (n=31), and after 2000 (n=91).

In the four studies, whole genomic DNA was extracted for further analysis of population genetic variability, differentiation and demographic history assessment. In Paper I, DNA was extracted using the Molestrips DNA tissue kit. In Paper III, DNA was extracted using salt extraction method modified from Aljabi and Martinez (1997) or the QIAGEN DNeasy Blood and Tissue Kit. The latter kit was also used for the DNA extraction of the muscles samples in Papers II and IV. For the museum European otter bone samples, in Paper IV, after being drilled into fine bones powder, DNA was extracted using a modified version of protocol C in Yang et al. (1998).

Molecular ecology markers
A broad scope of molecular markers have been developed and used for population analyses, including allozymes, RFLPs, AFLPs, microsatellites, SNPs as well as mitochondrial and nuclear DNA sequences. In the papers included in this PhD thesis, three types of markers were used for different applications and are described thereafter.

Allozymes
In Paper II, three allozyme loci (Pmi, Mdh-2, and Pgi-1) were used to detect molecular heterogeneity in the full moose sample (n=20,358). Allozymes are allelic vari-
ants of enzymes and were historically the first molecular markers broadly used to investigate diversity patterns within and among populations. The different alleles can be differentiated according to size and charge through gel electrophoresis (Sick 1961).

**Mitochondrial DNA sequences**

Regions of the mitochondrial DNA were amplified and sequenced in Papers II and III. In both these papers, a part of the control region of the mitochondrial DNA was sequenced. The control region contains hypervariable parts, which are often used to describe population genetic variability. In addition, in Paper II, a 365 bp fragment of the cytochrome oxidase I gene was sequenced. More details about the amplification, the purification of the PCR products can be found in the respective papers. The laboratory work for the mitochondrial DNA for Paper III was conducted at the Department of Bioinformatics and Genetics, Swedish Museum of Natural History. The laboratory work for Paper II was conducted at URS 3278, Perpignan, France. The sequences were edited and assembled using BioEdit (Paper II) and Geneious (Papers II, III).

**Microsatellites**

Microsatellite loci consist of short tandem repeats (1-6 bp long) that are found throughout the genome. Due to single-strand slippage during *in vivo* DNA replication, repeats can be added or lost. This slippage can occur at comparatively high rates leading to mutation rates ranging between $10^{-3}$-$10^{-5}$ mutations/locus/generation (Ellegren 2004). Thus, high levels of polymorphism can be found within species and microsatellites are thus particularly useful to describe population variability (Selkoe & Toonen 2006). Microsatellites were employed in Papers I, III and IV. In Paper I, nine microsatellites previously characterized for *Pararge aegeria* were used. In Paper III, twelve microsatellites were genotyped for *Alces alces* and in Paper IV, we genotyped twelve loci in the *Lutra lutra* samples. In the three papers, the amplifications were performed in multiplex PCR reactions using the QIAGEN multiplex PCR master mix. The grouping of the loci in the multiplex PCRs, the details of the fluorescence labeled primers and the reaction settings can be found in the respective papers. Capillary electrophoresis was conducted on an ABI 3130xl. The sizes of the fragments were determined using the GeneScan™ 500 LIZ™ (Papers I and IV) or the GeneScan™ 600 LIZ™ size markers (Paper III). For Paper I and Paper IV, the laboratory work and genotyping were performed at the Department of Bioinformatics and Genetics, Swedish Museum of Natural History. For Paper III, the laboratory work and the genotyping were performed at the Center of Evolutionary Application, University of Turku, Finland. In all three papers, genotypes were scored using GENEMAPPER v4.0 (Applied Biosystems).
Analytical methods

Genetic diversity
Levels of genetic diversity were investigating in all four papers. For the microsatellite datasets (Papers I, III and IV) and the allozyme dataset (Paper III), several statistics, such as the mean number of alleles, observed and expected heterozygosities, and allelic richness were used to describe variation within and among different sampling locations. Moreover, in Paper IV genetic diversities were also compared across different points in time. Genetic variation was also estimated for the mitochondrial DNA sequences (Papers II, III) by evaluating, for example, the number of haplotypes, haplotype diversity, as well as nucleotide diversity and the number of segregating sites among sequences.

Comparisons of populations and estimates of genetic structure
We investigated if and how the samples were genetically structured in space and in time. For several sampled areas, we assessed connectivity in terms of gene flow between sampling locations. For each marker, we were interested in examining differences in allele and haplotype frequencies and how these were distributed in space. The phylogenetic relationships between samples and geographic locations were inferred using tree-based methods. The relationships among mitochondrial DNA haplotypes were also estimated using minimum spanning or median-joining networks (Papers II and III). From the spatial distribution of alleles and haplotypes, we assessed the genetic divergence between populations using for example $F_{ST}$ or $\Phi_{ST}$ statistics (Wright 1951). Genetic and geographical distances were also compared using Mantel tests and tested for isolation by distance (Paper II). Population clustering was conducted using Analysis of Molecular Variance (AMOVA) or using individual-based approaches (i.e., spatial autocorrelograms, STRUCTURE, TESS).

Neutrality tests and population history inference
Fossil remains or museum collections are valuable in population genetics since they allow for direct estimates of changes in genetic diversity. However, when only contemporary samples are available, the assessment of past changes in population size is more challenging. Several statistical tests have been developed for DNA sequences and for multilocus microsatellite genotypes to detect departure from mutation-drift equilibrium, which can be used to infer demographic histories. For DNA sequences, Tajima’s $D$, Fu’s $F_S$ were computed and the results were interpreted in terms of past changes in effective population size. For microsatellites, heterozygosity-excess tests were performed to detect earlier population bottlenecks (Papers III, IV). Furthermore, coalescent-based simulations coupled with Approximate Bayesian Computation (ABC) were employed to infer the history of populations. The ABC-framework allows to assess more complex scenarios of evolution, selects the best scenario and estimates posterior distributions of model parameters by comparing summary statistics between observed and simulated datasets (Bertorelle et al. 2010; Csillery et al. 2010).
Summary of papers

Paper I

Postglacial recolonisation in the speckled wood butterfly

One of the most prominent features of the last ice age was the last glacial maximum period around 20,000 years ago. At that time, most of Britain and Northern Europe as far south as Germany and Poland were covered by the Scandinavian Ice Sheet. The ice started melting around 17,000 years ago allowing temperate plants and animals, which had been restricted to refugia, to recolonise the previously glaciated areas (Mangerud et al. 2011; Svendsen et al. 2004). In this study, we investigated the post-glacial recolonisation of the speckled wood butterfly, *Pararge aegeria*, in northern Europe using microsatellite genetic markers. We found an overall pattern of latitudinal decrease in allelic richness, which is consistent with the hypothesis that range expansions lead to successive losses in genetic variation due to repeated founder events (Hewitt 2004). Furthermore, using a Bayesian model-based clustering method, a marked population structure was detected. In the dataset, six genetic clusters were identified, corresponding to six geographically separate populations: [1] Central Scandinavia, [2] Gotland, [3] Öland, [4] South Scandinavia, [5] Benelux and [6] Eastern Baltic. Interestingly, previous studies have found very low genetic differentiation further south in Europe and in North Africa (Habel et al. 2013; Vandewoestijne & Van Dyck 2010). We hypothesized that the population structure observed in our study is a consequence of repeated founder effects during the post-glacial range expansion, since this type of process can lead to increased population divergence (Klopfstein et al. 2006; Ray & Excoffier 2009).

To further examine the recolonisation of northern Europe, we compared different postglacial range expansion models using an ABC approach, with emphasis on different scenarios for the origin of the population in Central Scandinavia (Fig. 1). We tested three plausible scenarios where Central Scandinavia was recolonised either from the south (South Scandinavia) or the East (Eastern Baltic). Among the three scenarios tested, we could reject the recolonisation of Central Scandinavia from the East. This means that the post-glacial recolonisation of northernmost Europe (Central Scandinavia and Eastern Baltic) took place along two routes, with one route on each side of the Baltic. This is interesting because *Pararge aegeria* displays different local adaptations in different parts of northern Europe, where populations in both Central Scandinavia and Eastern Baltic are univoltine (i.e. have one generation per year), while populations further south are multivoltine. Thus, under the assumption that the source populations in the south were multivoltine, as they are today, and that no gene flow has occurred between Central Scandinavia and Eastern Baltic, the ABC results suggested that univoltinism evolved independently on both side of the Baltic Sea.
The oceans cover approximately 70% of the earth’s surface, and have few obvious geographic barriers to dispersal. This, coupled with the pelagic larval stage displayed by many coral reef species implies that one might expect high levels genetic variation and little genetic differentiation among regions. On the other hand, declines in global sea levels during the last glaciation likely had a major effect on coral reef species, both because their distributions were more restricted and because the formation of land bridges may have reduced connectivity among populations. To investigate the demographic history and genetic structure in a widespread coral reef fish, we analysed genetic variation in the convict surgeonfish (*Acanthurus triostegus*) sampled across the Indo-Pacific. We recovered sequences from two mitochondrial DNA (mtDNA) markers, the left hypervariable domain of the control region and the cytochrome oxidase I gene.

High levels of haplotype and nucleotide diversities ($h > 99$ and $\pi = 8.9\%$) were found. Moreover, a lack of phylogeographic structure across the species range was revealed in the haplotype networks (Fig. 2). These results are consistent with a large long-term effective population size in *Acanthurus triostegus*, and likely also reflect the species’ capacity for long distance dispersal during its pelagic larval stage.
lar results have been observed for species with comparable wide geographic ranges (Gaither et al. 2010; Horne & van Herwerden 2013; Klanten et al. 2007).

In addition, it should be noted that significant genetic differentiation was observed for populations at the species range margin (e.g. Clipperton and the Marquesas). A high degree of differentiation has been commonly reported in these isolated regions, where the presence of cryptic species is known. This is also in accordance with the fact that the species richness is lower than in the west-Pacific and that a high degree of endemism have been reported for Marquesas (Lessios & Robertson 2006; Szabo et al. 2014; Williams et al. 2013)

To further explore the demographic history in *Acanthurus triostegus*, we used an ABC approach. The results from these analyses indicated that a ten-fold expansion in population size took place, roughly at the end of the last glaciation. This result is consistent with a hypothesis that climate-driven rises in sea levels at the end of the last glaciation may have led to re-arrangements in coral reef distributions. This may have had cascading effects on many fish species that rely on them. Interestingly, we also observed signatures of a more ancient, probably Middle Pleistocene, demographic expansion in the distribution of pairwise differences among the mitochondrial DNA sequences. Thus, our results revealed a complex demographic history that may be attributed to the sea-level fluctuations. Still, these findings also indicated the need of large sampling efforts combined with a multi-locus analysis to better address this complex demographic history. Moreover, to better understand the coral reef history of the Indo-Pacific, there is a need to perform comparative multi-species studies, based on different life-history traits.

![Minimum spanning network for *Acanthurus triostegus* sampled across the Indo-Pacific](image)

**Fig. 2**

Minimum spanning networks for *Acanthurus triostegus* sampled across the Indo-Pacific, based on 365 bp of mitochondrial CR sequences (n= 161: A) and based on 449 bp of mitochondrial COI sequences (n=179: B). Each circle represents a single haplotype and the circle size is proportional to the frequency of the haplotype. Each hatch-mark represents a nucleotide change. Colours indicate haplotype location.
Paper III

Autosomal and mitochondrial genetic variation in the Swedish moose

In Paper III, we investigated the demographic history and genetic structure in European moose (*Alces alces*) in Sweden. The European moose likely survived the Pleistocene glaciations in multiple refugia south or southeast of the Scandinavian ice sheet and recolonised Fennoscandia around 8000-9000 years ago (Haanes *et al.* 2011; Kangas *et al.* 2013; Niedziałkowska *et al.* 2014). These historical events have probably influenced the present genetic structure and diversity in Sweden. However, the moose in Scandinavia have also been strongly affected by more recent anthropogenic factors such as hunting and changes in landscape use. In particular, the Swedish moose population was severely reduced in the 19th and early 20th Centuries due to excessive hunting. Although the population has recovered, approximately one third (c. 100,000 animals) of the population is currently killed annually.

To assess to what extent present-day genetic patterns have been influenced by glacial dynamics as well as more recent human hunting, including the historical bottleneck, we examined genetic variation using allozymes and microsatellite markers in 20,000 and 1200 moose samples, respectively. To further examine genetic patterns potentially caused by the postglacial recolonisation of Scandinavia, we also sequenced the mitochondrial DNA control region in 48 moose samples. The autosomal markers demonstrated the existence of two major genetic groups, one in northern and one in southern Scandinavia, which were separated by a narrow transition zone (Fig. 3). Similar divisions into northern and southern groups have previously been observed in Finnish and Norwegian moose (Haanes *et al.* 2011; Kangas *et al.* 2013). Genetic divergence estimates in both autosomal and mitochondrial markers were comparatively limited among the two populations, suggesting that the populations diverged during the Holocene and consequently are unlikely to be the result of postglacial recolonisation from two separate glacial refugia. The ABC analyses indicated that both the northern and southern populations went through a bottleneck. The inferred timing of this bottleneck was consistent with the known bottleneck that took place from the 18th to the 20th Century.

At a finer geographic scale, we also found some evidence of additional substructure within the southern subpopulation. Moreover, spatial autocorrelation analyses suggested comparatively small “genetic patch sizes”. Thus, it appears that limited dispersal distances, estimated as only a few kilometers in our study, have led to a pattern of isolation by distance within the subpopulations.

From a management perspective, the two genetically distinct subpopulations identified in this study, need to be taken into account in order to ensure preservation of potentially unique genetic variation in the respective subpopulations. However, the estimated “genetic patch size” generally exceeds the size of current management areas, indicating that overharvesting in separate management areas would be unlikely to have any major genetic effects on the overall Swedish moose population.
Fig. 3
Color coded 3D surface plot of assignment probabilities to the northern (red) of the two major clusters identified by the software STRUCTURE, using the 1207 moose data set and 15 loci (12 microsatellites and 3 allozymes). The two major clusters and the transition zone previously identified in Norway are shown in three shades of grey (Haanes et al. 2011).

Paper IV
Recent demographic bottleneck in Eurasian otter from Sweden

Although European otters (Lutra lutra) used to be abundant across large parts of the Palaeartic, their population sizes started to decrease severely in the 1950s’ throughout most parts of Europe (Mason & Macdonald 1986). Polychlorinated biphenyls (PCBs) have been identified as one of the major drivers for the population decline (Mason 1993; Olsson & Sandegren 1991). However, even though several studies have examined the present-day genetic variation in otters (Hobbs et al. 2011; Mucci et al. 2010; Randi et al. 2003; Stanton et al. 2014), little is known about the genetic consequences of the bottleneck that took place in the 1950’s. In our study, using microsatellite data from historical as well as modern samples, we were able to test whether this bottleneck led to any loss in genetic diversity and/or changes in genetic structure. Comparisons of allelic richness at different points in time demonstrated a significant loss in diversity in southern Sweden (Fig. 4). In contrast, we found no evidence of declines in genetic diversity in northern Sweden.

Bayesian assignment of individual genotypes into genetic clusters indicated a pronounced genetic structure in both the modern and pre-bottleneck samples. Interestingly, historical and modern samples from northern Sweden were assigned to the same clusters, indicating that allele frequencies have remained stable through the last 60 years. However, historical and modern samples from southern Sweden were
assigned to different clusters, suggesting a marked change in allelic composition likely due to genetic drift. These results suggested that the bottleneck was more severe in southern compared to northern Sweden.

The results from ABC analyses further supported this pattern. A bottleneck scenario was supported for both northern and southern Sweden, and the inferred effective population sizes (Ne) during the height of the bottleneck were similar (~100). However, the posteriors for the post-bottleneck effective population sizes were highly different, where otters in northern Sweden appear to have recovered more (56% of the pre-bottleneck Ne) compared to in southern Sweden (17% of the pre-bottleneck Ne).

Overall, the genetic results fit well with a previous study on environmental toxin loads in otters, which demonstrated a higher concentration of PCBs in otters from southern Sweden compared to northern Sweden (Roos et al. 2001). Conservation efforts should take into account the observed pattern of genetic structure and careful consideration is required for the southern population, which may be particularly vulnerable.

Fig. 4
Rarefaction curves of allelic richness. (A) Estimates of allelic richness for subpopulations in northern Sweden. (B) Estimates of allelic richness for subpopulations in southern Sweden.
**Future directions**

This thesis illustrates how genetic tools can be used to reconstruct demographic histories. The studies encompassed a broad taxonomic diversity as well as a range of different environments, and made use of several different genetic markers. Past demographic changes were inferred both at long time scales to assess the consequences of climate change at the end of the last ice age (Papers I & II), as well as short time scales to examine the consequences of recent anthropogenic impacts (Papers III & IV). However, it should be noted that the accuracy in the inferences made in these studies sometimes are imprecise due to large confidence intervals in the estimated parameters. Moreover, the use of a limited number of loci can lead to incorrect inferences because gene trees do not always capture the true relationships among populations. Thus, to reiterate a declaration that likely has been around since the start of scientific time, more data is needed! Fortunately, recent technological advances now make this possible, moving the challenge to the analysis of the data.

**Recent developments in sequencing and computational technologies**

Today, with the emergence of next-generation sequencing (NGS) technologies, large-scale datasets and complete genomes have become more easily available. This is revolutionizing the fields of molecular ecology, conservation biology and population genetics (Ellegren 2014; Luikart *et al.* 2003). Thus, the field of population genetics is moving towards population genomics (Andrews & Luikart 2014) and more and more scientists talk about conservation genomics (Narum *et al.* 2013). Large SNP datasets can, for example, be obtained using restriction site-associated DNA sequencing (RAD-seq), and these may permit more complex demographic history to be revealed (Emerson *et al.* 2010; Lemmon & Lemmon 2013; Puritz *et al.* 2014; Reitzel *et al.* 2013).

However, to estimate complex demographic and historical effects on genetic variation (e.g. effective population size, gene flow, divergence), computer simulations are needed and these have started to play an increasingly important role (Hoban 2014; Hoban *et al.* 2011). Thus, genomics is to some extent dependent of developments in the bioinformatics field, where new simulators and programs with more sophisticated features emerge rapidly (Yuan *et al.* 2012). For example, the ABC framework, which is both powerful and flexible, has become especially popular to estimate demographic histories and other types of population genetic inference (Beaumont 2010; Beaumont *et al.* 2002; Bertorelle *et al.* 2010; Cornuet *et al.* 2014; Csillery *et al.* 2010; Wegmann *et al.* 2010). Additional recent developments for inference-based computational methods also include the simultaneous use of genetic data from several taxa to understand the interplay between, for example, geography, climate fluctuations and demographic change (Hickerson *et al.* 2007). These methods use a coalescent-based hierarchical ABC (hABC) framework. For example, MTML-msBayes allow testing of simultaneous divergence and migration across multiple co-distributed taxon-pairs (Huang *et al.* 2011). Another statistical develop-
ment based on hABC explores the demographic history of multiple taxa to detect concerted demographic expansions at the community-level (Chan et al. 2014).

Moreover, new methods continue to be developed to infer historical changes in effective population size. A recent major development uses the pairwise sequentially Markovian coalescent model (PSMC), which is based on the assumption that local densities of heterozygotes allow inference of the local time to the most recent common ancestor (Li et al. 2008; MacLeod et al. 2013). This method has the advantage that it enables inference of species’ population size histories based on single diploid genomes. This has for example been used to reconstruct the demographic history in several marine mammal species (Moura et al. 2014; Yim et al. 2014; Zhou et al. 2013), and with the aid of ABCs analyses can also help to identity ancient admixture (Miller et al. 2012) or to reconstruct divergence history (Nadachowska-Brzyska et al. 2013).

NGS and adaptive genetic variation
In addition to enabling more accurate inference population histories, large-scale genomic data can also be used to detect regions of the genome under natural selection, which in turn can be used to identify locally adapted traits among populations (Nielsen et al. 2005). For example, selective sweep mapping can be performed to detect purifying selection (Boitard et al. 2012; Foll & Gaggiotti 2008; Kim & Stephan 2002; Messer & Petrov 2013). Most of these tests are based on the genetic hitchhiking concept, where genome scans are employed to identify regions with local reduction in genetic diversity where purifying selection has acted. Moreover by integrating genomic and environmental datasets, potential ecological and environmental drivers of selection can be revealed (Jones et al. 2013; Joost et al. 2007; Schoville et al. 2012; Stapley et al. 2010).

Ancient DNA and museum collections
The recent development of the ancient DNA field can also provide new insights to understand how the past has affected the present. For example, the use of serially sampled ancient DNA data (Hadly et al. 2004) now permits demographic histories to be investigated in real-time as changes occurred, thus facilitating interpretations of the interaction between genetic and climatic changes (de Bruyn et al. 2011; Shapiro et al. 2004). The development of ancient DNA tools also highlights the utility of museum collections in genetic research. By allowing the comparison of historical and present-day genetic diversities, museum collections provide the opportunity to better understand the impacts of recent environmental change and human activities (Bi et al. 2013; Moritz et al. 2008; Nachman 2013; Ramakrishnan et al. 2005; Thomas et al. 1990; Wandeler et al. 2007). In Paper IV, we made use of this approach through sampling of both contemporary samples and museum specimens from Eurasian otters in Sweden. This allowed us to compare levels of genetic diversity and differentiation before, during and after a well-documented bottleneck.
**Palaeogenomics**
Coupled with the development of more advanced extraction and next-generation sequencing methods, the ancient DNA field has now entered the age of the palaeogenomics, pushing the limits of the DNA recovery (Millar & Lambert 2013; Orlando et al. 2013) and enabling new ways to calibrate molecular clocks as well as examining selection (Campbell et al. 2010; Fu et al. 2014; Shapiro & Hofreiter 2014). Another method that has recently been developed takes advantage of temporal sampling to investigate the footprint of genomic differentiation among samples to provide information about the populations’ histories. Thus, genetic differentiation between temporally spaced samples now make it possible to distinguish between (i) constant population size evolution, (ii) bottleneck models and (iii) replacement models, something which was not possible without taking into account the temporal sampling (Skoglund et al. 2014).

**A new genetic era**
To conclude, the fields of molecular ecology, population genetics and conservation genetics are being revolutionized by the fact that next-generation sequencing is resulting in a rapidly growing amount of data becoming available. More than ever, evolutionary biology is becoming a multidisciplinary field where archeologists, ecologists, geneticists, statisticians, and informaticians are collaborating. As a result, we can continue to satisfy our curiosity to understand the environmental, ecological and evolutionary processes that shape the genetic variation and biodiversity at different temporal and spatial scales.
Contributions

Paper I
VN performed the DNA extractions. VN and JLT conducted PCRs and genotyping. JLT conducted all the data-analysis with input from ES on the ABCs analysis. LD, KG and JLT wrote the manuscript.

Paper II
JLT performed all the laboratory work and analysed the data, with input from LD and SP. JLT wrote the manuscript with input from all coauthors.

Paper III
JLT performed the laboratory as well as computational analyses on the mitochondrial DNA. LW analyzed the allozyme and microsatellite data, except for the coalescent-based ABC analyses that were done by JLT. LW wrote the paper, with input from JLT and the other coauthors.

Paper IV
JLT collected and did the laboratory work on the museum otter samples. VB and PG performed laboratory analyses on the modern samples, under the supervision of JLT and EP. VB and JLT performed the computational analyses. VB wrote the paper together with JLT and LD.
References


Reitzel AM, Herrera S, Layden MJ, Martindale MQ, Shank TM (2013) Going where traditional markers have not gone before: utility of and promise for RAD


Såväl långsiktiga miljöförändringar, till exempel de som drivs av istidscykler, som mer nutida antropogent drivna förändringar har haft stor effekt på demografin hos vilda organismer. Inom en art speglas dessa förändringar genom mängden och den geografiska utbredningen av genetisk variation. I denna avhandling analyserades mitokondriellt- och mikrosatellit-DNA för att undersöka hur miljöförändringar i olika rums- och tidsskalor har påverkat genetisk variation och struktur hos fyra ekologiskt skilda djurarter.

Istidscyklerna anses ha spelat en stor roll i utvecklingen och fördelningen av arter. **Artikel I** undersöker den postglaciala rekoloniseringen av Kvickgräsfjärilen (*Pararge aegeria*) i norra Europa. En minskning av genetisk diversitet i förhållande till latitud och en tydlig populationsstruktur upptäcktes, vilket överensstämmer med en hypotes om att den postglaciala koloniseringsprocessen innebar ett flertal lokala flaskhalser (s.k. ”founder events”). Bayesianska beräkningsanalyser genom ”Approximate Bayesian Computation” (ABC) indikerade att de univoltina populationerna i Skandinavien och Finland härstammar från rekoloniseringar längs två vägar, en på var sida om Östersjön.

**Artikel II** syftade till att undersöka hur tidigare höjning av havsnivån påverkat populationen av Sebrastrimmig kirurgfisk (*Acanthurus triostegus*) i Indiska Oceangen och Stilla Havet. Inferens av artens demografiska historia indikerade en populations-expansion ungefär vid tiden för slutet på den senaste istiden. Därtill visade resultaten en övergripande brist på fyllogeografisk struktur, sannolikt på grund av den höga spridningsförmågan som artens pelagiska larvstadie innebär. Populationer i den Sebrastrimmig kirurgfiskens östligaste utbredningsområde var signifikant differentierade från andra populationer vilket sannolikt är en konsekvens av deras geografiska isolering.

I **Artikel III** analyserades människans effekt på den genetiska variationen hos den svenska älgstammen (*Alces alces*). Genetiska analyser påvisade en tydlig spatial struktur med två genetiska kluster, en i norra och en i södra Sverige, som var åtskilda med en transitionszon. Därtill indikerade demografisk inferens med hjälp av ABC-analys en recent flaskhals i populationsstorlek. Den uppskattade tidpunkten för denna flaskhals stämde väl överens med en känd minskning i älgstammen som skedde under 1800- och 1900-talen på grund av högt jakttryck.

I **Artikel IV** undersöktes effekten av en indirekt men välbeskriven mänsklig påverkan, den genom miljötoxiska kemikalier (PCB), på den genetiska variationen hos eurasisk utter (*Lutra lutra*) i Sverige. Genetiska klusteranalyser påvisade en differentiering mellan uttrar från olika delar av Sverige. ABC-analys indikerade att en minskning i populationsstorlek skett i både norra och södra Sverige. Jämförande analyser av historiska och nutida prov påvisade en kraftigare minskning av genetisk variation i södra jämfört med norra Sverige, vilket överensstämmer med de tidigare nivåer av PCB som uppmätts i respektive område.
Résumé en français

Les changements environnementaux à long terme, tels que ceux induits par les cycles glaciaires, et les impacts anthropiques plus récents ont eu des effets majeurs sur la démographie passée des organismes sauvages. Au sein des espèces, ces changements se reflètent dans la quantité et la distribution de la variation génétique neutre.

Dans cette thèse, l’ADN mitochondrial et des microsatellites ont été analysés pour quatre espèces animales écologiquement différentes, afin de déterminer comment des facteurs environnementaux et anthropiques ont affecté la diversité génétique et la structure des populations, à différentes échelles spatiales et temporelles.

Les cycles glaciaires sont considérés comme ayant joué un rôle important dans l’histoire et la distribution des espèces. L’article I décrit l’histoire de la recolonisation postglaciaire du papillon tircis (Pararge aegeria) en Europe du Nord. Une diminution de la diversité génétique corrélée avec la latitude ainsi qu’une forte structuration des populations ont été révélées. Ceci est compatible avec une hypothèse d’effets fondateurs répétés durant la recolonisation postglaciaire. En outre, les analyses d’inférences bayésiennes approximatives semblent indiquer que les populations univoltines (produisant une seule génération par an) en Scandinavie et en Finlande proviennent de recolonisations le long de deux routes distinctes, une route de chaque côté de la Baltique.

L’article II vise à étudier comment les variations du niveau des océans ont affecté l’histoire des populations du poisson chirurgien bagnard (Acanthurus triostegus) dans l’Indo-Pacifique. L’évaluation de l’histoire démographique de l’espèce a suggéré une expansion de la population qui a eu lieu autour de la fin de la dernière glaciation. De plus, les résultats ont démontré un manque global de structure phylogéographique, probablement en raison de taux élevés de dispersion pélagique au stade larvaire de l’espèce. Cependant, les populations à l’extrémité orientale de la zone de distribution de l’espèce sont significativement génétiquement différenciées des autres populations. Ceci est vraisemblablement une conséquence de leur isolement géographique.

Dans l’article III, nous avons évalué l’effet de l’impact humain sur la variation génétique des élans européen (Alces alces) en Suède. Les analyses génétiques ont révélé une structure spatiale avec deux groupes génétiques: un dans le nord et un au sud de la Suède, séparés par une étroite zone de transition. Par ailleurs, l’inférence démographique suggère un goulot d’étranglement de population récent, coïncidant avec une réduction de taille de la population connue au 19ème siècle et au début du 20ème siècle en raison d’une pression de chasse élevée.

Dans l’article IV, nous avons examiné l’effet d’un impact humain indirect mais bien décrit, celui des produits chimiques toxiques environnementaux (PCBs), sur la variation génétique des loutres eurasiennes (Lutra lutra) en Suède. Les analyses d’affectation individuelle en groupement génétique ont révélé des populations distinctes de loutres dans le nord et le sud de la Suède, mais aussi dans la région de
Stockholm. Les analyses d’inférence bayésienne approximative ont indiqué une diminution de la taille effective des populations à la fois dans le nord et le sud de la Suède. De plus, des analyses comparatives d’échantillons historiques et contemporains ont démontré une baisse plus sévère de la diversité génétique dans le sud de la Suède par rapport au nord de la Suède, en accord avec les différents niveaux de PCBs trouvés dans ces régions.