

Evolutionary and conservation biology of the Finnish house sparrow

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Academic dissertation

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- II** Kekkonen, J., Hanski, I.K., Väisänen, R.A. & Brommer, J.E.: More heavy metals in urban house sparrows compared to rural ones in southern Finland. — Manuscript
- III** Kekkonen, J., Hanski, I.K., Jensen, H., Väisänen, R.A. & Brommer, J.E.: Increased genetic differentiation in house sparrows after a strong population decline: From panmixia towards structure in a common bird. — Manuscript
- IV** Kekkonen, J., Jensen, H. & Brommer, J.E.: Mixed patterns of drift and selection working on Finnish house sparrows. — Manuscript

Contributions

The following table shows the major contributions of authors to the original articles or manuscripts.

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Summary

Recently it has been recognized that evolutionary aspects play a major role in conservation issues of a species. In this thesis I have combined evolutionary research with conservation studies to provide new insight into these fields. The study object of this thesis is the house sparrow, a species that has features that makes it interesting for this type of study. The house sparrow has been ubiquitous almost all over the world. Even though being still abundant, several countries have reported major declines. These declines have taken place in a relatively short time covering both urban and rural habitats. In Finland this species has declined by more than two thirds in just over two decades. In addition, as the house sparrow lives only in human inhabited areas it can also raise public awareness to conservation issues.

I used both an extensive museum collection of house sparrows collected in 1980s from all over Finland as well as samples collected in 2009 from 12 of the previously collected localities. I used molecular techniques to study neutral genetic variation within and genetic differentiation between the study populations. This knowledge I then combined with data gathered on morphometric measurements. In addition I analyzed eight heavy metals from the livers of house sparrows that lived in either rural or urban areas in the 1980s and evaluated the role of heavy metal pollution as a possible cause of the declines.

Even though dispersal of house sparrows is limited I found that just as the declines started in 1980s the house sparrows formed a genetically panmictic population on the scale of the whole Finland. When compared to Norway, where neutral genetic divergence has been found even with small geographic distances, I concluded that this difference would be due to contrasting landscapes. In Finland the landscape is rather homogeneous facilitating the movements of these birds and maintaining gene flow even with the low dispersal.

To see whether the declines have had an effect on the neutral genetic variation of the populations I did a comparison between the historical and contemporary genetic data. I showed that even though genetic diversity has not decreased due to the drastic declines the populations have indeed become more differentiated from each other. This shows that even in a still quite abundant species the declines can have an effect on the genetic variation. It is shown that genetic diversity and differentiation may approach their new equilibriums at different rates. This emphasizes the importance of studying both of them and if the latter has increased it should be taken as a warning sign of a possible loss of genetic diversity in the future.

One of the factors suggested to be responsible for the house sparrow declines is heavy metal pollution. When studying the livers of house sparrows from 1980s I discovered higher levels of heavy metal concentrations in urban than rural habitats, but the levels of the metals were comparatively low and based on that heavy metal pollution does not seem to be a direct cause for the declines in Finland. However, heavy metals are known to decrease the amount of insects in urban areas and thus in the cities heavy metals may have an indirect effect on house sparrows.

Although neutral genetic variation is an important tool for conservation genetics it does not tell the whole story. Since neutral genetic variation is not affected by selection, information can be one-sided. It is possible that even neutral genetic differentiation is low, there can be substantial variation in additive genetic traits indicating local adaptation. Therefore I performed a comparison between neutral genetic differentiation and phenotypic differentiation. I discovered that two traits out of seven are likely to be under directional selection, whereas the others could be affected by random genetic drift. Bergmann's rule may be behind the observed directional selection in wing length and body mass. These results highlight the importance of estimating both neutral and adaptive genetic variation.

1. Introduction

Biodiversity can be measured at three different levels: the community or ecosystem level, species level and genetic level (Pullin 2002). Since not all things in nature can be directly measured or observed, genetic methods have given new insights into history, present status and future estimates of species (Awise and Hamrick 1995, deSalle and Amato 2004). Genetic tools provide means to monitor changes in populations over time (Schwartz et al. 2007). They can produce information relevant to both ecological and evolutionary time scales, while often being cheaper and sometimes more sensitive and reliable than traditional monitoring approaches. Also in some cases monitoring cannot even be done with other methods (Schwartz et al. 2007). Through genetic monitoring information can be gained on for example species abundances (e.g. Piggot et al. 2006, rock-wallaby), vital rates (e.g. Rudnick et al. 2005, Eastern imperial eagle), geographical range of the species (e.g. McKelvey et al. 2006, Canada lynx) and hybridization (e.g. Hitt et al. 2003, trout species) as well as demographic and evolutionary processes. The latter two can be estimated by determining changes in population size with measures of allelic diversity, expected heterozygosity (Spencer et al. 2000), allele frequencies (Luikart et al. 1999) or effective population size (e.g. Palm et al. 2003, brown trout). Also measuring the connectivity and gene flow between populations has become important (Koskinen et al 2002, Coulon et al. 2006).

It has been recognized that the current state of a species is a result of constant change and the future of the species also depends on the evolutionary processes. Accordingly, in modern conservation biology the scope has shifted from protecting species within static communities to conserving systems that are dynamic (Pullin 2002). Conservation concerns or actions are often focused on species that are rare and thus in a great risk of extinction (Soulé 1987). However, species which are still fairly common but declining also prompt some conservation concerns. The International Union for Conservation of Nature (IUCN) criteria for the conservation status of organisms take into consideration abundant species if their declines meet the criteria of e.g. $\geq 30\%$ decline in ten years

or three generations (qualifies to status Vulnerable) (IUCN Red List Categories and Criteria version 3.1). Loss of individuals is one of the mechanisms through which genetic diversity is lost (Frankham 1996, Hartl and Clark 2007). Thus the global scale IUCN criteria and country-specific conservation definitions of policy – which aim at preventing the declines – help to protect this key aspect of species' viability. Preserving a sufficient level of genetic diversity maintains the potential for a species or population to adapt to changing environmental conditions (Spielman et al. 2004, Frankham et al. 2009).

1.1. Genetic diversity as a key to species existence and evolution

Environments are under constant change. In the course of evolution, species change according to the surrounding conditions they experience. Especially today, when humans alter the globe at a high rate, the need for species to adapt to changes in their environments has become more critical (Frankham 1999). For populations to adapt to environmental changes, genetic diversity is required. Genetic diversity is the variation in alleles and genotypes in the species or population. It serves as the raw material for adaptive evolutionary change (Frankham et al. 2002). In a genetically diverse species different forms are maintained and when the optimum of the species shifts due to changes in their environment, forms that are beneficial in the new circumstances are being selected for and they become more common. If there is little diversity, the new optimum may not be reached and the species is less adapted to its environment and consequently in a greater risk for extinction.

Genetic diversity is generated by mutations, which create new alleles into the gene pool. Migration maintains diversity by enabling gene flow between populations. Also balancing selection maintains diversity. Directional selection on one hand creates locally adapted forms, but on the other hand divides the gene pool and in the process diversity can also be lost. Genetic drift has the potential of eradicating diversity by chance while acting in a random manner on the genetics of the species (Hartl and Clark 2007). Species and their

populations are simultaneously affected by all of these evolutionary forces which can also work to counteract each other. The relative roles are being determined by e.g. population size: in large populations selection is predominant whereas the effects of drift and gene flow become more pronounced in small populations. The direction of evolutionary change is thus determined by the interactions of these forces (Maynard Smith 2000).

1.2. Consequences of declines in genetic diversity

Large populations of outbreeding species usually have extensive genetic diversity. When abundance declines it affects the genetic patterns of the species. In small populations, the relative role of random genetic drift as evolutionary force increases, which can lead to loss of alleles and heterozygosity (Frankham et al. 2002, Hartl and Clark 2007, Amos and Balmford 2001). Along with the alleles also the number of heterozygotes declines resulting in a reduced fitness. Analyses in a review by Reed and Frankham (2001) indicate that there is a significant overall relationship between population mean heterozygosity and population fitness. It has been noted recently, however, that the methods used in some of these heterozygosity-fitness correlation -studies (HFC) have not been optimal. This has been actively discussed and good practices have been specified (Chapman et al. 2009, Szulkin et al. 2010). Nevertheless, in general the relationship of decreased fitness due to reduced heterozygosity still holds.

Studying genetic diversity alone may not reveal the whole picture. Crow and Aiko (1984) and Varvio et al. (1986) showed with simulation studies that the new equilibrium level of genetic diversity is reached in a much slower rate than the genetic differentiation between diverging units. Thus, it is important to investigate both genetic diversity and genetic differentiation in population genetic studies with conservation implications. If the species is geographically structured and the numbers decline, the populations become smaller and more isolated from each other. This means that less migrants move between the populations resulting in less gene flow which would maintain the diversity (Avice and Hamrick 1995). In small

populations, the effects of random genetic drift become more pronounced and can counteract the effects of natural selection which, by chance, can lead to strong decline of genetic diversity, loss of evolutionary beneficial alleles and fixation of harmful alleles (Amos and Balmford 2001). This in turn can lead to situations where the populations are even more vulnerable to harmful environmental changes as well as stochastic processes that fluctuate more in small than large populations. Consequently small populations can more easily reach a point from which they can not recover anymore.

Conservation studies most often concern species that are rare and in greatest risk of extinction (Soulé 1987). However, it is also important to examine species that are declining but still relatively abundant. These species may raise conservation concerns in the future and on the other hand they can increase knowledge on mechanisms and consequences that decreasing numbers have on the genetic diversity of a species. These things are not always possible to study on threatened species and thus this information could be applied to their conservation as well (Avice and Hamrick 1995).

1.3. Temporal studies revealing changes in genetics

The most powerful approach for linking a population decline to a change in the population genetics relies on temporal comparisons (Schwartz et al. 2007). Analyzing specimens collected prior to the decline can open possibilities to still reconstruct the past and give measures comparable to contemporary ones (Payne and Sorenson 2002, Leonard 2008). Some reference values can be inferred from a closely related species or from the same species in a different area, but most reliable results are gained if pre- and post-decline data from the same population are used. If low genetic diversity is observed in the contemporary data, it can be evaluated whether it actually is a result of the decline (e.g. Hector's dolphin, Pichler and Baker 2000) or present already in historical samples indicating that low diversity is a species-specific feature (e.g. Morro Bay kangaroo rat, Matocq and Villablanca 2001). Some studies regarding birds have compared historical and contemporary

samples (reviewed in Wandeler et al. 2007). Variable results have been found: decrease in allelic richness (Bouzat et al. 1998) or heterozygosity (Groombridge et al. 2000), increased genetic differentiation but no loss of heterozygosity (Martinez-Cruz et al. 2007) and no effect at all (Brown et al. 2007). However, it is noteworthy that most of these studies consider species that have gone through a severe bottleneck. However, the IUCN conservation criteria apply also in case a major decline occurs in a species that remains relatively common. Also in these situations it would be interesting to see whether genetic diversity is lost despite still being abundant in number and how well are population genetic changes detected? Here, genetic methods for population monitoring become especially important because populations can go through so called “cryptic bottlenecks” in which the bottleneck is not detected through traditional demographic monitoring methods but appears in genetic studies (Luikart et al. 1998).

When comparing pre- and post-decline populations, possible changes in genetic diversity indices, such as allelic richness, private allelic richness and expected heterozygosity can be measured. In addition, estimating the level of spatial differentiation of populations before and after the reduction in population size gives valuable information on whether populations have become more isolated due to restricted gene flow (Schwartz et al. 2007, Wandeler et al. 2007, Leonard 2008). Temporal estimates of effective population sizes are also valuable. Effective population size indicates the number of individuals that actually produce offspring and it thus predicts the extinction risk better than mere population size. Unfortunately, it is often difficult to estimate (Wang 2005). Furthermore, temporal studies enable the detection of new introductions and assessing the rate of genetic introgression into indigenous populations (Wandeler et al. 2007).

1.4. Selection vs. genetic drift as evolutionary forces

In conservation studies, the level of genetic differentiation is often measured using markers which are not under selection. While valuable information is gained through this approach, in order to

have a more comprehensive understanding of the organisms’ genetic attributes, it is important to perceive how different evolutionary forces act on the populations and try to infer into what directions these forces might take them in the future. A way to study the relative roles of random genetic drift and natural selection is to measure both neutral and adaptive genetic variation in the same samples from a set of populations and compare the levels of differentiation. Neutral variation is most often determined as F_{ST} (Wright 1943, Nei 1987) and variation in quantitative traits, assumed to be under selection, as Q_{ST} (termed by Spitze 1993). In this comparison there are three possible outcomes (Merilä and Crnokrak 2001, McKay and Latta 2002). If $Q_{ST} > F_{ST}$, natural selection is favoring different phenotypes in different populations and thus driving directional selection. If the two measurements are equal, it can not be ruled out that the observed differentiation would have been caused by genetic drift alone. If $Q_{ST} < F_{ST}$, same phenotypes are favored in different populations and stabilizing selection is occurring. Reviews by Merilä and Crnokrak (2001) and Leinonen et al. (2008) suggest, that in most cases adaptive genetic variation exceeds the neutral expectation indicating that directional selection is acting as the main evolutionary force. Nevertheless, also contrary findings have been observed (e.g. Lee and Frost 2002, Baruch et al. 2004, Evanno et al. 2006) and because there might be a bias towards finding directional selection instead of genetic drift (Miller et al. 2008, Whitlock et al. 2008) more studies are needed in this field.

1.5. Human associated species at risk – the case of the house sparrow

As humans alter the globe in accelerating rate some organisms benefit from the changes but many suffer. Species that have become associated with human settlements and thrived with growing human population have had to face in the past few decades also the adverse effects of modern urbanization. One such species is the house sparrow (*Passer domesticus*) which has spread around the world with humans. This species has been highly successful and abundant but recently in many countries it has been reported to decline dramati-

cally (Anderson 2006). These declines have taken place in both rural and urban habitats, but different causes may be responsible for the declines in these different habitats. Also because the species is considered sedentary, urban and rural populations may not always mix through migration and consequently individuals in different habitats could differ. For example Bjorklund et al. (2010) found in great tits (*Parus major*) in Barcelona that genetic variation was larger in the parks compared to the forest population and gene flow was higher from the town to the forest than the other way around. Studies on the causes of house sparrow declines should take the possible habitat-related differences into consideration. In general lack of nest sites (Siriwardena et al. 2002) and lack of food for both adults and nestlings (Hole et al. 2002, Summers-Smith 2003, Vincent 2005) as well as predation, inter-specific competition (McCarthy 2003) and diseases (Kruszewicz 1995) have been suggested to cause the wide-scale house sparrow declines. In particular in urban areas the role of pollutants like e.g. heavy metals has been speculated (Summers-Smith 1999). In reality the cause for declines may be a combination of some or all of these. The close relationship to humans has been a stepping stone for the house sparrow to spread all over the world, but nowadays it may turn out to be the stumbling block. The changes in human lifestyle over the past century may have just occurred too rapidly for the house sparrow to adapt to.

1.6. Aims of the thesis

House sparrow is an interesting species to use in an evolutionary and conservation studies because of its a) recent sharp declines in Finland as well as in other parts of the world, b) strong human association and c) sedentary nature. In this thesis I sampled data from present house sparrow populations in Finland and compared them with a unique and extensive museum collection from the pre-decline period of this species. The aim of the first chapter (I) was to establish the levels of genetic diversity and population structure for house sparrow males just as the declines started in Finland and the species was still highly abundant. In the second chapter (II) liver samples from the museum collection were used to conduct a heavy metal analysis. The aim was to study how heavy metals

accumulated to birds living in either urban or rural habitats, were there sex-dependent differences or did the heavy metals accumulate with age. In chapter three (III) the objective was to test for changes between pre- and post-decline datasets to see whether genetic population differentiation had increased or within-population genetic diversity had decreased due to the declines. In chapter four (IV) the aim was to utilize the morphometric measurements taken from birds sampled after the declines and use them together with the neutral genetic marker data to study the relative roles of natural selection and random genetic drift. All in all in this thesis I focused on gathering novel information on this dramatically declined but still rather abundant species by using multiple approaches to gain as inclusive picture of its population genetics as possible.

2. Species and Methodology

2.1. The house sparrow

House sparrow is one of the most familiar birds to humans in many parts of the world. It is strongly human associated and even though it is found from small isolated farms to multimillion metropolises, it is basically absent from uninhabited areas. Because of this close relationship to man it has invoked feelings of both love and hatred and has been consequently treated as either a companion or nuisance (Anderson 2006, Summers-Smith 2006). In agriculture the house sparrow has been treated as a pest, eating crops and spreading diseases and has been eradicated accordingly. However, many farmers also see this species as a part of the wildlife in their backyard.

The house sparrow is a sedentary species. The limited dispersal is mainly undertaken by juveniles dispersing from their native areas. Females are more prone to disperse than males. (Summers-Smith 1988, Skjelseth et al. 2007). Long-distance recoveries from Britain and Ireland showed that only 3% of dispersal events extend further than 20 km (Siriwardena et al. 2002). In Norway only 10% of female and 6% of male fledglings that were later recruited to the breeding population had left their native islands (Altwegg et al. 2000). Furthermore, Tufto et al. (2005) found that most

house sparrows did not disperse from their natal farms, and of the ones that did, 90% dispersed less than 36 km. Despite the low dispersal rates, the natural distribution range of the house sparrow covers Europe, Northern Africa and much of Asia. Furthermore, humans have introduced this species either accidentally or intentionally to North America, South America, Australia, New Zealand and parts of Africa (Summers-Smith 1988). Along with human settlement, house sparrows have successfully spread to these new areas and quickly naturalised.

House sparrows are social animals, which gather in flocks for many activities such as feeding (Summers-Smith 1988). They also tend to breed in small colonies. Nests are placed preferably in natural cavities or buildings, but also in nest boxes, trees or even shrubs. House sparrows mainly feed on seeds and grains of natural herbs or cultivated crops. In urban areas house sparrows have also learned to exploit the leftovers and garbage of humans. Although the quality of this type of food is probably worse (Crick *et al.* 2002), this flexibility has been one key to house sparrow's success in the cities. During the breeding season the availability of invertebrates is important as they serve as the sole food for chicks during the first days after hatching (Summers-Smith 1988).

Because of its adaptability, the house sparrow has been highly successful and abundant. In recent decades, however, this species has suffered from major declines, which have puzzled both scientists and the general public. Declines have been reported in large parts of Europe (Summers-Smith 1999, Hole *et al.* 2002, Crick *et al.* 2002, Anderson 2006, de Laet and Summers-Smith 2007), as well as in areas where it is non-native, such as North America and India (Peterjohn *et al.* 1994, Goyal 2005). Despite the declines, this species is considered as Least Concern (LC) on the global IUCN red list criteria, because it is still rather abundant and its distribution range is large. In Finland, just prior to the declines started in early 1980s, abundance of house sparrow was estimated at 400,000 pairs (Väisänen *et al.* 1998). Since then this species has declined by over 60% in just two decades. Declines have taken place in both rural and urban habitats but they have been slightly more severe in urban areas (Väisänen 2003; Figure 1). When the Finnish criteria for the conservation status of spe-

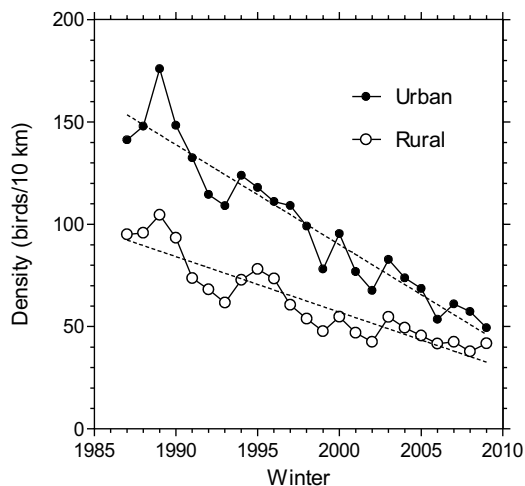


Fig. 1. Density (birds per 10 km of transect) of the house sparrow in Finnish winter bird censuses in urban and rural habitats during 23 winters 1986/1987–2008/2009. Predicted from linear regression (hatched lines) the species decreased on average 70% in urban and 65% in rural areas.

cies were updated in the year 2000 (Rassi *et al.* 2001), the house sparrow was classified as Near Threatened (NT). Given its huge and rapid declines, the species could have been classified as more endangered, if strict application of IUCN criteria was applied, but it was left at this more cautious level because of its high relative abundance compared to many other species and the expectation that the declines would be halted (Rassi 2000). Furthermore, in the last renewal in 2010 (Mikkola-Roos *et al.* 2010), the house sparrow was taken of the list altogether, based on its presumed stabilization in population size.

2.2. Data collecting and analysis

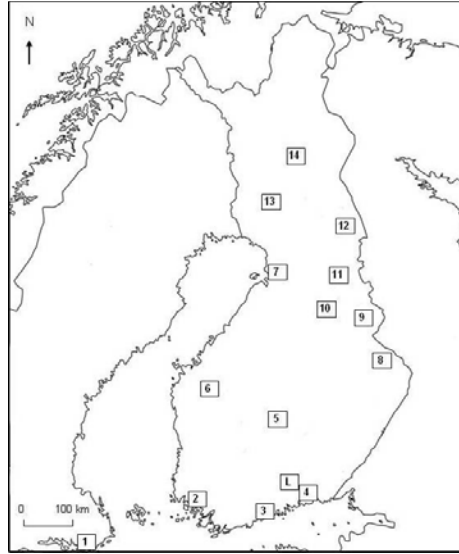
Ringling records

Ringling records from 1930–2004 used in chapter I were obtained from the Ringling Bureau, Finnish Museum of Natural History, Helsinki.

Pre-decline samples

Pre-decline samples were collected during 1983–1986 from all around Finland and also from

Fig. 2. Map of Finland representing the sampling localities of house sparrows indicated by numbers from 1 to 14. Names of sampling localities are indicated besides the figure. There are thirteen sampling sites in Finland and one in Stockholm, Sweden.



Names of sampling localities:

- 1 Stockholm
2. Turku
3. Helsinki
4. Myllykoski (Lahti=L)
5. Jyväskylä
6. Seinäjoki
7. Oulu
8. Lieksa
9. Kuhmo
10. Kajaani
11. Ämmäsaari
12. Kuusamo
13. Rovaniemi
14. Sodankylä

Stockholm (Sweden). Fourteen localities were included in chapter I (Figure 2) and all together 472 house sparrow males were used in the study. For chapter III twelve of these localities were used so that Stockholm and Seinäjoki were excluded from the original fourteen populations. Sample size was 705 birds (sexes combined). In different chapters the numbering of the localities is always started from 1.

Samples for chapter II were chosen from the collection in a finer scale from southern and central Finland. Five places were located in urban areas: four in the very centre of the capital city Helsinki and one in the city of Jyväskylä, central Finland. In addition, house sparrows were sampled in three rural areas outside the capital area (Figure 3). Sample size added up to 56 house sparrows.

Birds were sampled with permission by catching with mist nets and killed with carbon dioxide. Subsequently the birds were measured, sexed, aged and then dissected and preserved in the natural history museum. Livers were preserved at -18°C . They were used as the source of DNA and for heavy metal analysis in this thesis.

Post-decline samples

Post-decline samples were collected from ten localities in February 2009. In addition samples were provided by ringers from Turku and from

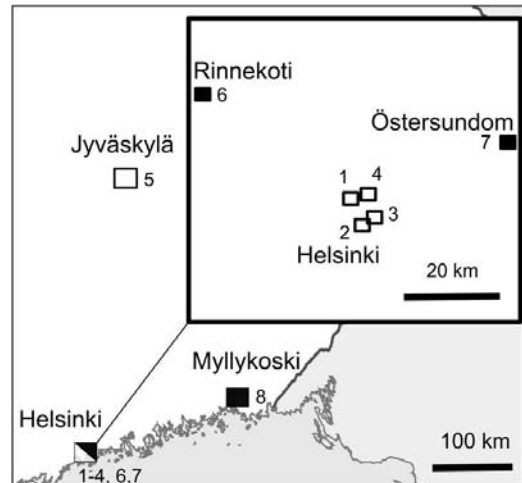


Fig 3. Map of southern and central Finland where sampling sites are indicated with numbers from 1 to 8 (filled squares=rural, unfilled squares=urban).

Lahti in 2008. The latter locality was 60 km north-west from the locality Myllykoski sampled in the 1980s and was considered representative for this site (noted in Figure 2 with L). Seinäjoki and Stockholm were excluded from data gathering. Sampling was attempted at the same locations that were used for the sampling the pre-decline samples taken in the 1980s. However, in the post-decline sampling, differences may have occurred because for some localities the information of the exact locations of sampling had been lost and for

some places the sampling methods defined the possible catching sites. However, this does not likely pose problems, because in this geographical scale small shifts should not matter. For chapter **III** 281 samples were used from the twelve locations. For chapter **IV** two localities (Turku and Lahti) were excluded and sample size was 238 individuals.

In ten localities, house sparrows were caught with mist nets and a small blood sample (70ul) was taken for DNA analysis. In addition, tarsus length, bill length, bill depth from both sexes and visible badge size and total badge size from males with a sliding caliper with an accuracy of ± 0.1 mm were measured. Total badge size was measured including all the feathers on the throat that were either black or with black bases and grey tips. Visible badge size was measured by including only totally black feathers. I measured body mass with a Pesola spring balance (± 0.1 g) and wing length with a ruler (± 0.5 mm). In addition, feather sample from birds caught in the Turku and Lahti were obtained. In all the above sampling schemes, birds were ringed, which allowed to verify that no individuals were sampled twice.

Genetic data: Microsatellite genotyping

Microsatellites are parts of non-coding DNA with tandem repeats of 1 to 6 bases. In diploid organisms the two homologous chromosomes can have either two identical or two different repeat numbers in the same locus. Microsatellites are co-dominant, usually selectively neutral and often highly polymorphic markers due to their high mutation rate and thus suitable for population genetic studies (Goldstein and Schlötterer 1999, Ellegren 2000). Microsatellites were used in chapters **I**, **III** and **IV** and genotyping was conducted in the same way in all of them, only the source of DNA differed. Liver samples were used in chapter **I**. In chapter **III** liver, blood and feather samples were used and in chapter **IV** blood samples were utilized. The following thirteen microsatellite loci were analyzed in all of these chapters: Pdo μ 16, Pdo μ 19, Pdo μ 22, Pdo μ 27, Pdo μ 33, Pdo μ 40, Pdo μ 44 and Pdo μ 47 (Dawson et al. in review), Ase18 (Richardson et al. 2000), Pdo μ 1 and Pdo μ 3 (Neumann and Wetton 1996), Pdo μ 10 (Griffith et al. 2007), and Pdo μ 5 (Griffith et al. 1999).

Heavy metal analysis

The heavy metal analysis was conducted in the laboratory of the Finnish Environment Institute (SYKE). The laboratory is accredited by the international standards and the methods and equipment are certified. Levels of eight heavy metals were analyzed as mg kg^{-1} dry weight from the liver samples: aluminium (Al), chrome (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb). The analytical procedure was based on US EPA (1994) –method 3051A “Microwave Assisted Acid Digestion of Sediments, Sludges, Soils and Oils” with some modifications.

Statistical analysis

In chapters **I** and **III** the genetic diversity within the populations was described with three parameters: (1) allelic richness (A_R), (2) expected heterozygosity according to Hardy-Weinberg equilibrium (H_E) (program FSTAT 2.9.3, Goudet 2001) and (3) private allelic richness (A_{PR}) (program HP-RARE, Kalinowski 2005). Inbreeding coefficient (F_{IS}) was also calculated by using FSTAT. The level of genetic differentiation among the populations was assessed as F_{ST} (Wright 1943) by using the Weir and Cockerham’s (1984) θ estimator as implemented in the software FSTAT 2.9.3 (Goudet, 2001). F_{ST} was estimated both globally and between all pairs of populations. A Mantel’s test was used to test for isolation by distance, i.e. correlation between the level of genetic differentiation and geographic distance. Also in chapters **I** and **III** the software STRUCTURE 2.2 (Pritchard et al. 2000a, Falush et al. 2003a and 2007) was used to cluster populations into groups based on individual genetic data without any prior information on geographic sampling locality. Individuals were also assigned to populations using GENECLASS 1.0.02 (Cornuet et al. 1999). In addition programs BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) and Arlequin 3.11 (Excoffier et al. 2005) were used to test whether study populations had gone through a decline in population size in recent history.

In chapter **III** additional analysis were performed to study whether the population differenti-

ation has changed after the declines. First, a separate analysis of F_{ST} from pre- and post decline data was ran in program R 2.10.1 (R development Core Team 2010) with package Hierfstat (Goudet 2006), which gave confidence intervals for global F_{ST} values. If the confidence intervals did not overlap between the two time periods the difference was considered significant. Second, Arlequin 3.11 (Excoffier et al. 2005) was used to perform an analysis of molecular variance (AMOVA) on the combined data, where pre-decline and post-decline samples that were taken from one population formed one spatial group in the analysis. The AMOVA tested how the total variation in the genotypic data was explained by a) variance among spatial groups, b) variance among pre- and post-decline samplings within the spatial groups and c) variance within the samplings (each sampling taken from a population in one point of time). Hence, a significant variance between the time periods (i.e. b) indicated a change in genetic differentiation over time.

In chapter II Program SPSS Statistics 17.0 (SPSS for Windows, Rel. 17.0.0.2008, Chicago: SPSS Inc.) was used in all the statistical analyses of the data. The analysis that are described below were conducted for each heavy metal separately as well as using principal component analysis (PCA). A general linear model (GLM) was used to analyze the data. Either the individual heavy metals measured from the house sparrow livers or the principal components derived from them were used as the dependent variable. In each of these GLM's habitat, age and sex were included as fixed variables. Also possible interactions between these explaining variables were analyzed.

In chapter IV R package Hierfstat (Goudet 2006) was used for calculating the global F_{ST} values. The 95% confidence intervals were obtained by bootstrapping 10,000 times. Package MCMCglmm for R (Hadfield 2010) was used to produce posterior distributions of variance matrices where also effects of sex and age and their interaction were taken into account. Based on the posteriors, P_{ST} and its credible interval (95% highest posterior density) was approximated for various parameters that scale the estimated phenotypic to the additive genetic variances as

$$P_{ST} = \frac{c \sigma_B^2}{c \sigma_B^2 + 2h^2 \sigma_W^2} = \frac{\frac{c}{h^2} \sigma_B^2}{\frac{c}{h^2} \sigma_B^2 + 2 \sigma_W^2}, \quad (1)$$

where σ_B^2 stands for the phenotypic variance between populations, σ_W^2 denotes the phenotypic variance within populations and h^2 the heritability (the proportion of phenotypic variance that is due to additive genetic effects). The scalar c expresses the proportion of the total variance that is presumed to be due to additive genetic effects across populations. That is, when $c = 1$, all variation across population is assumed to be additive genetic and when $c = 0$ all variance across population is due to environmental (or other non additive-genetic) effects. Since both h^2 and c are in this case unknown and the end result is dependent on their reciprocal ratio, the equation is modified such that they are form essentially one parameter (c/h^2). Figures were drawn to illustrate the change in approximate P_{ST} values and its credible interval for each trait when c/h^2 varied. In interpreting the $P_{ST} - F_{ST}$ relationship, we focused around the point where trait specific heritability estimate (h^2) equaled c . This was considered as a realistic point estimate around which the effects of increasing or especially decreasing c/h^2 would be biologically most relevant to examine. Population means for the traits that indicated between population differentiation were plotted against latitude with program SPSS 17.0 (SPSS for Windows, Rel. 17.0.0.2008, Chicago: SPSS Inc.).

3. Results and Discussion

3.1. House sparrows population genetics before and after the declines

Dispersal ability, geographic variation, level of genetic diversity, level of genetic differentiation and adaptation to local conditions are key aspects of a species' ecology (Frankham et al. 2002, Hartl and Clark 2007). When aiming to compare the present state of a species with some earlier point in time molecular tools come handy. Even though we can not go back in time to observe the species, we can derive information using e.g. museum specimens as a source of genetic material (Wandeler et al. 2007). In this thesis I have aimed at gaining im-

Table 1. Genetic differentiation (F_{ST} estimates) among population pairs. Below the diagonal are F_{ST} values from the old samples and above the diagonal are the values from new samples. Significant F_{ST} values after sequential Bonferroni correction are in bold. Number of genotyped individuals are presented in Table 1. Note that sample size after the decline was much lower than prior to the decline resulting in a higher critical F_{ST} value.

	Turku	Helsinki	Myllykoski	Jyväskylä	Oulu	Lieksa	Kuhmo	Kajaani	Ämmänsaari	Kuusamo	Rovaniemi	Sodankylä
Turku	–	0.0062	0.0073	0.0042	0.0135	0.0222	0.0150	0.0117	0.0253	0.0069	0.0099	0.0167
Helsinki	0.0025	–	0.0027	0.0002	0.0126	0.0142	0.0090	0.0087	0.0288	0.0053	0.0072	0.0075
Myllykoski	0.0028	0.0033	–	–0.0030	0.0075	0.0182	0.0110	0.0041	0.0202	0.0060	0.0134	0.0153
Jyväskylä	0.0016	0.0026	0.0024	–	0.0124	0.0143	0.0128	0.0084	0.0256	0.0051	0.0092	0.0067
Oulu	0.0003	0.0026	0.0037	0.0025	–	0.0213	0.0180	0.0076	0.0307	0.0176	0.0130	0.0163
Lieksa	0.0066	0.0093	0.0029	0.0047	0.0060	–	0.0177	0.0209	0.0430	0.0202	0.0169	0.0156
Kuhmo	0.0061	0.0088	0.0037	0.0053	0.0074	0.0087	–	0.0152	0.0358	–0.0005	0.0105	0.0192
Kajaani	0.0035	0.0023	0.0001	0.0011	0.0020	0.0035	0.0057	–	0.0267	0.0145	0.0129	0.0126
Ämmänsaari	0.0086	0.0110	0.0099	0.0077	0.0084	0.0114	0.0102	0.0084	–	0.0293	0.0286	0.0410
Kuusamo	0.0039	0.0061	0.0035	0.0032	0.0036	0.0067	0.0069	0.0029	0.0063	–	0.0075	0.0096
Rovaniemi	0.0032	0.0052	0.0041	0.0034	0.0033	0.0078	0.0082	0.0038	0.0092	0.0045	–	0.0105
Sodankylä	0.0039	0.0077	0.0065	0.0072	0.0035	0.0124	0.0109	0.0058	0.0087	0.0066	0.0048	–

portant information on house sparrows by using molecular methods and two data sets sampled at different times from the same localities. In chapter I the level of population differentiation was established in 14 house sparrow populations before the species declined in Finland. This is also linked to the effects of landscape composition i.e. how uniform landscape can enhance gene flow. In chapter III this was extended by analyzing pre- and post-decline samples to compare the situation before and after the declines.

Panmictic population structure before the declines – effects of landscape composition

In chapter I we found from the Finnish ringing records, that only 10% of juveniles dispersed further than 16 km. Hence, the available ringing records suggest that natal dispersal of Finnish house sparrows is highly limited. Furthermore adult dispersal ($n = 1110$) was also expectedly low i.e. median was 0 km and only 10% dispersed more than 3 km. The low dispersal detected suggests that populations may also be spatially structured but this does not necessarily translate into genetic differentiation between populations. Indeed, we found that the levels of genetic differentiation (measured by F_{ST}) were very low both in pairwise comparisons (Table 1) and globally (F_{ST} among

Finnish populations = 0.004 ± 0.001 (SE)), meaning that the populations formed basically one panmictic unit. Additionally, the low level of genetic differentiation was corroborated by the following findings: there was no isolation by distance (Mantel $r = 0.0000$, $p = 0.941$), all the Finnish sampling localities formed one cluster in the clustering analyses (posterior probability for $K=1$ was $\ln P(D) = -26\,659.8$, which was better than for $K=2$ for which $\ln P(D) = -26\,971.4$) and only 24.7% of individuals could be assigned to their population of origin. However, interestingly the Stockholm population was significantly differentiated from all Finnish populations, with F_{ST} values ranging between 0.023 and 0.038. Genetic diversity within house sparrow populations was very similar in all localities, including Stockholm.

Despite the low dispersal indicated by the ringing data, the house sparrow populations showed hardly any spatial structuring across Finland (about 400×800 km). Extensive homogeneity of populations was most likely due to populations being very large and possibly also better connected (at least until recent times) than ringing data suggested. At the time of sampling, the house sparrow was highly abundant in Finland, the census population size of the whole country being in the order of 10^6 individuals and distribution covering most human-inhabited areas from small farms to cities (Väisänen et al. 1998). In such populations, genetic drift has almost no effect and diver-

Table 2. Results from analysis of molecular variance (AMOVA). Variation is divided between among spatial groups, among pre- and post-decline samplings within spatial groups and within samplings (each sampling taken from a population in one point of time) components. P-value is obtained from 1,023 permutations.

Source of variation	Variance components	% of total variance	P-value
Among spatial groups	0.00228	0.04157	0.0186
Among pre- and post-decline samplings within spatial groups	0.04434	0.80773	0.0000
Within samplings	5.44253	99.15069	
Total	5.48915	99.9999	

gence is counteracted by gene flow. Dispersal of the house sparrows may also have been more frequent and may have covered longer distances than previous ringing records have suggested. On the other hand, only a small number of migrants are enough to even homogenize populations (Franklin 1980, Frankel & Soulé 1981, Allendorf 1983) and thus only few dispersal events may maintain connectivity in an unobstructed landscape. We concluded that landscape in Finland could have been considered rather contiguous for the house sparrow in the 1980s and house sparrows could have maintained gene flow over large geographical distances by a stepping-stone pattern of small migration distances in a fairly homogeneously distributed population.

Despite the low differentiation on the scale of Finland (the longest distance between sites 813 km), we also found that the distance of 250 km between Turku (Finland) and Stockholm (Sweden), including c. 40 km of open sea, was sufficient to create significant genetic differentiation among the populations. This is in line with other studies of house sparrow populations on the coast of Norway, where populations are separated by fjords and mountains (Bjordal 1986 and Jensen et al. in prep). These results underline the strong effect that landscape composition can have on dispersal; a species that in a contiguous landscape forms a homogenous population on the scale of hundreds of kilometers can have rather strong spatial structuring on a much smaller scale when barriers (such as open sea) limit dispersal.

From panmixia towards structure

Preserving sufficient level of genetic diversity maintains the potential for a species or population

to adapt to changing environmental conditions (Frankham et al. 2002). Since by losing individuals also genetic diversity is lost (Hartl and Clark 2007) studying the genetic diversity and population genetic structure before and after the declines is of great importance. Even though the house sparrow is still relatively abundant, this approach is relevant because the loss of genetic diversity may still impair its long-term survival and hamper its return to higher abundance (e.g. Hutchinson 2003). In chapter I house sparrows were found to be panmictic in Finland before the declines. With respect to the severity of the declines, signs of them could have been found in the genetics, but on the other hand the time scale between pre- and post-decline data was short. In chapter III we showed that the genetic population structure had indeed increased threefold globally (pre-decline $F_{ST} = 0.005$, post-decline $F_{ST} = 0.015$) and even though it still remained at a low level (Hartl and Clark 2007) it was significantly higher than before. The pairwise F_{ST} -estimates had also increased (Table 1), but because of smaller sample sizes the critical value for significance was twenty times higher. Furthermore, in analysis of molecular variance (AMOVA), the proportion of variation in the data was higher between the two time periods than between the geographical areas where the two temporal samples were combined (Table 2). Also more (36.3%) of individuals from the post-decline sample set could be assigned correctly to their population of origin compared with the pre-decline data (27.1%). This suggests that despite the contiguous landscape, the reduced number of dispersers resulted in lower gene flow between the populations. To be sure that the observed increase of genetic differentiation or the higher variation between time periods than geographical areas (from AMOVA) were not just due

Table 3. Basic population-level statistics of genetic variability: allelic richness (A_R), private allelic richness (A_{PR}), expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}). Garza-Williamson index indicated in rightmost column. Information is given for the pre-decline ("pre") and post-decline ("post") samples for each population. Note that post-decline samples in subpopulation three were taken from Lahti, a locality c. 60 km away from the original sampling locality Myllykoski. The statistics for "Total" are calculated for the all the populations pooled.

Population	AR	APR	HE	FIS	G-W-index
1. Turku pre	6.97	0.09	0.840	0.015	0.68
Turku post	6.74	0.03	0.819	-0.002	0.61
2. Helsinki pre	6.84	0.10	0.839	0.006	0.72
Helsinki post	6.88	0.11	0.840	0.041	0.62
3. Myllykoski pre	7.13	0.10	0.846	0.030	0.65
Lahti post	6.92	0.17	0.847	-0.016	0.58
4. Jyväskylä pre	6.90	0.06	0.839	0.017	0.70
Jyväskylä post	6.86	0.05	0.835	-0.021	0.62
5. Oulu pre	6.82	0.07	0.832	0.042	0.66
Oulu post	6.98	0.05	0.850	0.025	0.63
6. Lieksa pre	6.93	0.09	0.843	0.018	0.68
Lieksa post	6.73	0.18	0.832	0.083	0.53
7. Kuhmo pre	6.92	0.09	0.833	0.015	0.67
Kuhmo post	6.93	0.11	0.835	0.042	0.62
8. Kajaani pre	6.99	0.09	0.843	0.014	0.66
Kajaani post	6.97	0.18	0.839	0.035	0.59
9. Ämmänsaari pre	6.84	0.12	0.833	0.021	0.63
Ämmänsaari post	6.21	0.23	0.793	0.007	0.57
10. Kuusamo pre	6.98	0.06	0.844	0.009	0.67
Kuusamo post	6.70	0.07	0.822	0.030	0.61
11. Rovaniemi pre	7.07	0.06	0.852	0.024	0.64
Rovaniemi post	6.94	0.21	0.833	-0.017	0.58
12. Sodankylä pre	6.92	0.08	0.840	0.055	0.65
Sodankylä post	6.61	0.08	0.828	0.013	0.59
Total pre	16.31	1.45	0.844	0.025	
Total post	17.07	1.87	0.842	0.032	

to the smaller sample sizes in post-decline data, a resampling was done from the pre-decline data. The number of individuals that were present in the populations of the post-decline data was randomly sampled from the pre-decline samples and these analyses were conducted again. The results remained the same giving further support to the finding that house sparrow populations have become more differentiated due to the declines.

Given that the declines have taken place during approximately 12.7 generations (assuming a generation time of 1.96 years as documented by Jensen et al. 2008), it is actually surprising that we found evidence of change in the population genetic structure. Little more than ten generations is not a long time for populations to reach new equilibrium states of diversity and differentiation. In

addition, the number of house sparrows has not dropped as low as classical examples of bottlenecked avian populations (e.g. Bouzat et al. 1998, Muñoz-Fuentes et al. 2005, Glenn et al. 1999 and Groombridge et al. 2000).

The fact that we did find significantly smaller expected heterozygosity and allelic richness as well as larger private allelic richness in the northern part of Finland after the declines (Table 3), can be explained by population demography. Even though the declines have not been more severe in the north (Väisänen, unpublished data) there were smaller population sizes of house sparrows in the northern part of Finland prior to the decline (which is at the edge of the species' distributional range) and as a consequence, the declines have had a proportionately stronger effect on the genet-

ics of these smaller populations. Furthermore, there was not strong evidence that any of the populations (including those in northern Finland) had gone through a bottleneck and the overall genetic structure was still fairly weak. To exclude the effect of smaller sample sizes in post-decline data these analyses were also repeated with the resampling from pre-decline data. The results did not change and to conclude, we did not find evidence that this decrease in genetic variability up north would have been caused by more severe declines.

Similarly to this study Martinez-Cruz et al. (2007) found a clear increase of genetic structure in Spanish imperial eagle populations, but did not find any decrease in the genetic diversity indices. However, this kind of pattern is explainable by among- and within-population components of variation changing at different rates. Crow and Aoki (1984) as well as Varvio et al. (1986) demonstrated with simulation studies that in recently fragmented populations genetic differentiation reaches the new equilibrium state much faster than within-population diversity. Based on this, the observed increase in genetic differentiation in this study should be taken as a cautious indication that even though the situation seems still fairly good, a loss of genetic diversity due to the declines may be ongoing in the Finnish house sparrows but will be detected only later on.

3.2. Heavy metals as a potential cause of house sparrow declines

Especially in urbanized areas harmful substances are produced in such amounts that they can have deteriorating effects on the development, survival and reproduction of organisms. Organisms, which have become closely associated to human, are at greatest risk of being affected. This can easily apply to the house sparrow and one hypothesis of the declines of the house sparrows has been heavy metal pollution, especially in the cities. In chapter II this issue is considered in a study where levels of eight heavy metals in the livers of urban and rural house sparrows in southern and central Finland were analyzed. As shown in Figure 1 in the declines in Finland have been slightly steeper in urban habitats.

Table 4. Concentrations of eight heavy metals (mg kg⁻¹) in livers of house sparrows from rural and urban habitats. Values represent mean \pm standard error.

Heavy metal	Rural	Urban
Al	0.21 \pm 0.14	2.70 \pm 1.83
Cr	0.23 \pm 0.03	0.18 \pm 0.02
Mn	1.25 \pm 0.07	1.39 \pm 0.06
Fe	429.50 \pm 19.93	563.61 \pm 20.71
Cu	3.69 \pm 0.17	4.03 \pm 0.15
Zn	18.35 \pm 0.75	21.08 \pm 0.75
Cd	0.09 \pm 0.01	0.16 \pm 0.02
Pb	0.10 \pm 0.02	0.54 \pm 0.06

Accumulation of heavy metals with age and differences between sexes

In the livers of Finnish house sparrows, the concentration of iron (Fe) was highest, with a mean of 515.71 mg kg⁻¹, followed by zinc (Zn) with 20.11 mg kg⁻¹. Other metals occurred with much smaller concentrations (smaller than 4 mg kg⁻¹; see Table 4). The concentrations were higher in urban birds in all heavy metals except chrome (Cr) (Table 4). Principal component analysis reduced the eight original variables to three independent principal components, which explained 43.1%, 16.0% and 13.5% of the total variance. Different metals had the following roles in results: iron (Fe), cadmium (Cd) and lead (Pb) accounted for most of the explained variance by PC1, chrome (Cr), copper (Cu) and zinc (Zn) in PC2 and aluminium (Al) and manganese (Mn) in PC3.

Interestingly, no metal accumulation was found when age was associated with PC1 (Table 5). Also PC2 and PC3 were not affected. Only in cadmium (Cd) there was an indication of higher level in older birds ($F_{1,54} = 3.71$, $p = 0.060$). On contrary to these results Swaileh and Sansur (2006) did find accumulation with age in some heavy metals in house sparrow livers as well as other parts of the body. However, in their study Swaileh and Sansur classified 1 to 4 week old sparrows as juveniles and the rest as adults. Heavy metals accumulated along the juvenile stages and were lower than in the adult group. In this study juveniles were several months old and our findings thus suggest that heavy metal levels quickly reach the same concentration in juveniles than in

Table 5. Result from general linear model (GLM) where principal component 1 was used as dependent variable and habitat, sex and age were explanatory variables. Interactions were found to be non-significant.

Source of variation	df	Sum of squares	F	p-value
Habitat	1	21.35	38.62	<0.001
Age	1	0.51	0.91	0.344
Sex	1	1.89	3.41	0.071

adults. However, some of the juveniles were sampled in the spring when some previous season offspring could potentially have fully ossified skulls. It is therefore possible that some of our adult birds were actually juveniles, which would further act to reduce the difference between the age groups.

Even though no accumulation with age was found, there was an indication that sexes differ in their accumulation of heavy metals in PC1 (Table 5). With PC1 males seemed to have higher levels of heavy metals. In PC2 and PC3 there were no effects of age. From separate heavy metals only zinc (Zn) gave nearly significant results with respect to sex ($F_{1,54} = 3.70$, $p = 0.060$). Also other studies on birds have indeed found differences with respect to sex (Burger 1991, Eeva 2009). Eeva et al. (2009) suggested that due to their higher reproductive effort females might be more susceptible to the negative health effects of pollution stress. Another possible reason would be that because of differences in dispersal, sexes would have experienced different environments as young. Even though the house sparrow is considered to be a sedentary species, females are more likely to disperse from their native areas than males (Summers-Smith 1988, Skjelseth et al. 2007). Since heavy metals do not move very far from the source point and differences in their levels may occur already in rather short distances (see e.g. Janssens et al. 2003), there might be proportionally more individuals in the female segment of the urban population that have dispersed from less polluted rural areas than in the male segment. Whether differences in heavy metal concentration between the sexes are expected in this particular species remains unclear; e.g. Swaileh and Sansur (2006) did

not find differences between the sexes in their study.

Heavy metal pollution in urban and rural habitats

In the general linear model where PC1 was the dependent variable, habitat was explaining most of the variation in the levels of metals and was highly significant (Table 5). Urban birds had more heavy metals accumulated in their livers (Figure 4). When analyzing the heavy metals separately, habitat explained significant amount of variation in iron (Fe) ($F_{1,54} = 15.73$, $p < 0.001$), zinc (Zn) ($F_{1,54} = 6.10$, $p = 0.017$), cadmium (Cd) ($F_{1,54} = 9.90$, $p = 0.003$) and lead (Pb) ($F_{1,54} = 31.12$, $p < 0.001$). Even though our results show clearly that urban house sparrows experience a different environment than rural birds with respect to accumulation of heavy metals, it is difficult to say to what extent these differences impact the population. Since the amounts we found were comparatively small, it is not likely that heavy metal pollution alone would have caused house sparrow declines in urban areas in Finland. This is supported by the fact that heavy metal emissions have also decreased during the house sparrow declines. However, because all studies to date (including ours) show that heavy

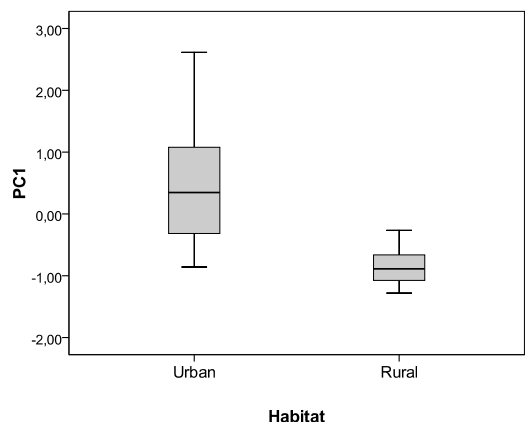


Fig 4. Box plots representing principal component 1 in different habitats. The horizontal line in the box represents the median, the hinges are the lower and upper quartiles and the lines are 1.5 times the interquartile range. Possible outliers that are outside this range are indicated with dots.

metal concentrations are higher in urban areas, it seems possible that heavy metals could play some role through other mechanisms. Besides the direct physiological effects, heavy metals can have indirect effects and this has been recently uplifted by house sparrow studies from Leicester, UK (Vincent 2006, Peach et al. 2008). Heavy metals along with other pollutants decrease the amount of invertebrates in the cities which are used as food for the house sparrow nestlings. Vincent (2006) found annual productivity (as number of fledged young) to be lower in urban areas due to starvation of chicks when their diet contained a high proportion of vegetable material or ants instead of for example spiders. In addition lower post-fledging survival was predicted based on nestling weights. Peach et al. (2008) reported that years of poor reproduction were characterized by – among other things – high concentration of air pollution from traffic. This together with removed vegetation seemed likely to decrease invertebrate availability in urban areas. To conclude, heavy metals may have indirectly played a part in the declines of urban house sparrows in Finland, but probably there are also other unknown factors that are together responsible.

3.3. Comparing quantitative trait divergence with neutral genetic differentiation

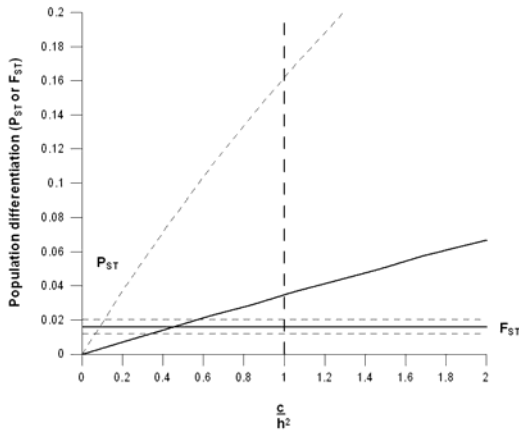
As neutral genetic divergence measures differentiation in genes that are not under natural selection, important information of evolutionary processes affecting the species can be lost, if only neutral variation is studied. Even with low neutral divergence, there can be substantial differentiation in quantitative genetic variation leading to locally optimized phenotypes (Leinonen et al. 2008). Being able to predict into what directions the evolutionary processes may modify the species is very important in terms of e.g. conservation (Leinonen et al. 2006, Palo et al. 2003). Therefore, in chapter IV roles of drift and selection were studied using neutral and quantitative variation.

Roles of drift and selection in shaping Finnish house sparrow populations

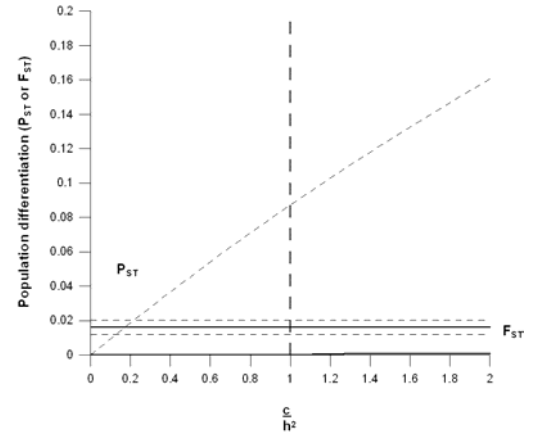
By comparing phenotypic trait variation to neutral genetic variation in several morphometric traits of the house sparrow we found that in tarsus length, bill depth and bill length the point estimate for approximate P_{ST} was lower than global F_{ST} , but the upper 95% credible intervals clearly encompassed the F_{ST} values (Figure 5A, 5C, 5D). This was the case also for the male traits of visible and total badge size (Figure 5F, 5G). Body mass and wing length were the only traits that showed evidence of significant differences between approximate P_{ST} and F_{ST} (Figure 5B, 5E). When considering the $c/h^2 = 1$ as a biologically realistic assumption where the proportion of variation due to additive genetic effect across populations c equals the proportion within population h^2 (indicated by a vertical dashed line in Figure 5), the global P_{ST} value for wing length was 0.062 and for body mass 0.212, which both were significantly higher than F_{ST} . For wing length, however, the evidence for $P_{ST} > F_{ST}$ was rather weak, because decreasing the proportion of additive genetic variance between populations only a little (c/h^2 ratio from 1 to 0.85), resulted in rapidly losing the significant difference between the neutral and adaptive divergence (Figure 5B). For body mass, on the other hand, the evidence for $P_{ST} > F_{ST}$ was stronger, because the ratio of c/h^2 would have to be less than 0.2 (more than five times lower proportion of phenotypic variance across populations than within populations are explained by additive genetic effects) for P_{ST} to decrease to the level of F_{ST} (Figure 5E).

In their review, Leinonen et al. (2008) found that in 70% of studies directional selection was responsible for the observed differentiation. However, few studies have compared quantitative and neutral divergence in birds (Leinonen et al. 2008). Phenotypic adaptation was found on European wide scales in greenfinches *Carduelis chloris* (Merilä et al. 1997) and great snipes *Gallinago media* (Saether et al. 2007) for morphometric traits and in pied flycatchers (*Ficedula hypoleuca*) for plumage coloration (Lehtonen et al. 2010). In great snipes, substantial phenotypic differentiation was found between Norway and Eastern Europe, despite low neutral divergence, but not between populations within the same region. Inter-

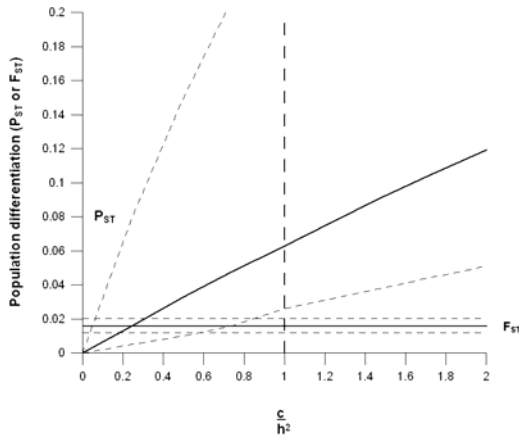
A Tarsus length



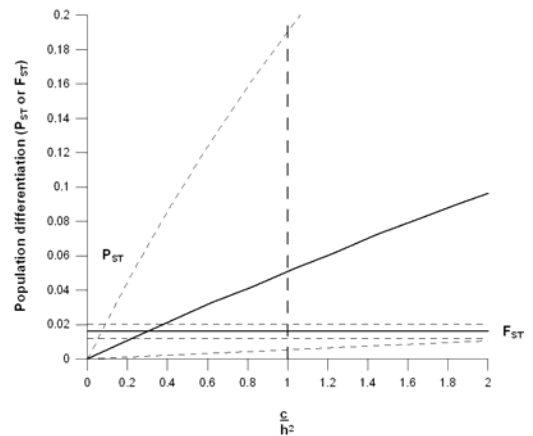
C Bill depth



B Wing length



D Bill length



estingly, we found evidence of quantitative divergence on the scale of just few hundred kilometers, suggesting that adaptation to local conditions due to diverging selection can be found in birds even on a relatively small spatial scale. Partly, this finding may reflect the more sedentary lifestyle of the house sparrow in comparison to the great snipe (migratory), greenfinch (at least partly migratory) and pied flycatcher (migratory). Although the limited dispersal of the house sparrow (Altwegg et al. 2000, Siriwardena et al. 2002, Tufto et al. 2005) has not resulted in large neutral divergence (our F_{ST} is 0.0159), it still seems to have restricted gene flow enough for local adaptation to occur. Furthermore, our findings were quite similar to those made in a recent study of house sparrow diver-

gence of the same traits in Norway (Holand et al. in review). There was a strong indication of directional selection for body mass. Tarsus length, wing length, bill depth and bill length showed somewhat similar patterns. In male visible and total badge size, however, quantitative trait differentiation exceeded neutral genetic differentiation in Norway but not in Finland. However, in our study sample sizes were smaller regarding these male traits.

Since directional selection on body mass and (to some extent) on wing length was detected, the population means of these two traits were plotted against latitude (Figure 6A, 6B). In both plots the traits seem to grow when moving to north, albeit for body mass there are some inconsistencies. For

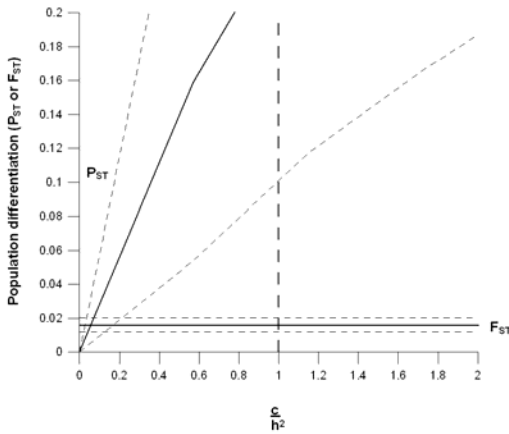
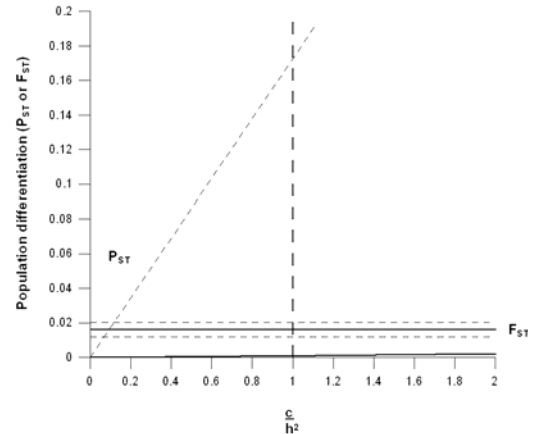
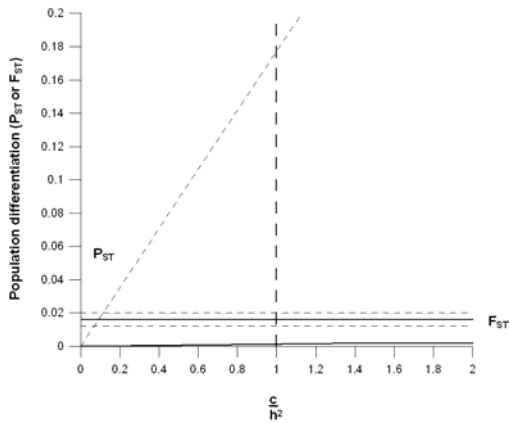
E Body mass**G Total badge size****F Visible badge size**

Fig. 5. The relationship between global P_{ST} and F_{ST} estimates (solid lines) for house sparrows when the ratio of the scalar parameters c (proportion of phenotypic variance between populations due to additive genetic effects) over heritability h^2 (proportion of phenotypic variance within populations due to additive genetic effects). The dotted lines represent the lower and upper 95% credible and confidence intervals of the P_{ST} and F_{ST} estimates respectively. Vertical dashed line represents the point in the x-axis where the value of c equals the heritability estimate h^2 (additive genetic effects explain an equal proportion of phenotypic variance between and within populations), which is here considered as a naive expectation. Each panel represents a different trait (named in the panel).

wing length the linear regression was significant ($b = 0.469 \pm 0.114$ SE, $p = 0.003$, Figure 6A) and but for body mass not quite ($b = 0.429 \pm 0.226$ SE, $p = 0.095$, Figure 6B). These traits appear to follow the Bergmann's rule (Bergmann 1847), which states that individuals experiencing cooler climate are larger in body size. Bergmann's rule is considered to be driven by directional selection and it is not expected if traits are influenced by environmental effects only. For further support to our findings, Finnish house sparrows in 1980s have been found to be under directional selection over winter (Väisänen and Hanski unpublished data). This has generally been quite well established for

birds since e.g. Ashton (2002) included studies conducted on 100 bird species to a meta-analysis and found out that for 76 of the species the Bergmann's rule held true. Interest in this ecogeographic rule has recently increased, because climate warming predicts shifts in the latitudinal gradients (e.g. Yom-Tov 2001).

From a conservation perspective this study showed that despite the still rather low neutral divergence there seems to be potentially adaptive divergence. The relevance of locally adapted forms for species' genetic diversity is however difficult to evaluate and further research is thus needed.

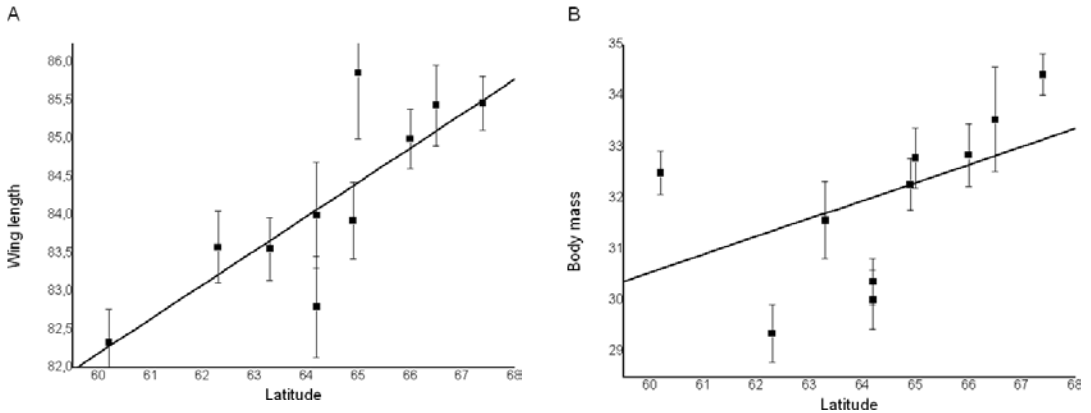


Fig. 6. Population means of wing length (panel A) and body mass (panel B) plotted against latitude. Error bars around the mean represent the standard errors. Latitude is given as degrees and the range corresponds to 60°N to 67°30'N.

4. Conclusions and the future of the house sparrow

In Finland the house sparrow population has been panmictic in 1980s. Due to the dramatic declines, however, population structuring has increased, even though the effect on loss of genetic diversity may lag behind. Despite this still rather low neutral divergence, directional selection was found to have acted on two morphometric traits, wing length and body mass, indicating local adaptation. Heavy metal pollution, which has been suggested as a possible cause of declines in urban areas, might partly explain the faster declines in cities than in the rural areas in Finland but through indirect effects like availability of insect food.

The house sparrow in Finland is still relatively abundant but the declines may not be over yet (Chapter III, Figure 1). At this moment I think that the future of this bird seems decent in Finland. If the abundance would stay approximately at the present level, the sheer numbers can potentially counteract the negative effects of population declines. I want to stress, though, that it is possible we have not seen all the effects of the declines yet, especially if new equilibrium states in the genetic diversity or differentiation have not been reached. In addition if the species continues to decline, the future prospects for the species can deteriorate also at the genetic level.

Overall, I think that even though conservation actions seem not to be needed at the moment, the case of the house sparrow can nonetheless provide

information relevant for modern conservation biology. Chapter I shows that genetic approaches provide information that is not gained by traditional monitoring methods: ringing records indicated low dispersal and based only on that populations could be expected to differ significantly. However, microsatellite data showed that house sparrows were panmictic. Chapters I and III emphasize the importance of genetic methods as tools for conservation studies since in a species that comes out from traditional censuses as relatively abundant, effects of the declines can already be detected in the genetics. In addition the importance of genetic monitoring also in the future becomes apparent since all effects may not have been seen so far. Findings from chapter II indicates that urban and rural birds are likely to experience different challenges in their living environments and this should be taken into consideration when studying declines in other human associated species. Chapter IV highlights the importance of using several genetic approaches in this type of population genetic research. Looking at the increased but still rather low neutral genetic divergence important information on differentiation in quantitative traits under selection would have been missed. Even though the results should not be over-interpreted, the $P_{ST} - F_{ST}$ -comparisons in species that are declining may present considerable insights that are applicable in designing evolution-based conservation strategies. The studies in this thesis could be extended in the future by including both historical and new samples from

other countries in Europe to study the effects of declines on a larger scale and whether the evolutionary processes differ between areas as they seem to when comparing these results to the Norwegian study. Also using markers for genes that code fitness related traits under selection would extend the understanding of evolutionary processes ongoing in the house sparrow.

References

- Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008). Genetic effects of harvest on wild animal populations. *Trends of Ecology and Evolution* 23: 327–337.
- Altwegg RT, Ringsby TH, Saether B-E (2000). Phenotypic correlates and consequences of dispersal in a metapopulation of house sparrows *Passer domesticus*. *Journal of Animal Ecology* 69: 762–770.
- Amos W, Balmford A (2001). When does conservation matter? *Heredity* 87: 257–265.
- Anderson TR (2006). *The Ubiquitous house sparrow: from genes to populations*. Oxford University Press.
- Ashton KG (2002). Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* 11 (6): 505–523.
- Avise JC, Hamrick JL (1995). *Conservation genetics, case histories from nature*. Chapman & Hall, New York.
- Baruch Z, Nassar JM, Bubis J (2004). Quantitative trait, genetic, environmental, and geographical distances among populations of the C4 grass *Trachypogon plumosus* in Neotropical savannas. *Diversity and Distributions* 10: 283–292.
- Bergmann C (1847). Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien* 3 (1): 595–708.
- Björdal H, Cole SR, Parkin DT (1986). Genetic differentiation among some populations of the House Sparrow, *Passer domesticus*, from southwestern Norway. *Heredity* 105: 107–114.
- Björklund M, Ruiz I, Senar JC (2010). Genetic differentiation in the urban habitat: the great tits (*Parus major*) of the parks of Barcelona city. *Biological Journal of the Linnean Society* 99: 9–19.
- Bouzat JL, Levin HA, Paige KN (1998). The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. *American Naturalist* 152: 1–6.
- Brown JW, Van Coeverden de Groot PJ, Birt TP, Seutin G, Boag PT, Friesen VL (2007). Appraisal of the consequences of the DDT-induced bottleneck on the level and geographic distribution of neutral genetic variation in Canadian peregrine falcons, *Falco peregrines*. *Molecular Ecology* 16: 327–343.
- Burger J, Gochfeld M. (1991). Cadmium and lead in common terns (*Aves: Sterna hirundo*): Relationship between levels parents and eggs. — *Environmental Monitoring and Assessment*. 16: 253–258.
- Chapman JR., Nakagawa S, Coltman DW, Slate J, Sheldon BC (2009). A quantitative review of heterozygosity-fitness correlations in animal populations. *Molecular Ecology* 18: 2746–2765.
- Cornuet JM, Luikart G (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001–2014.
- Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999). New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153: 1989–2000.
- Coulon A, Guillot G, Cosson J-F, Angibault JMA, Aulagnier S, Cargnelutti M, Galan M, Hewison AJM (2006). Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology* 15: 1669–1679.
- Crick H, Siriwardena G (2002). *National trends in the breeding performance of House Sparrows Passer domesticus*. Investigation into the causes of the decline of Starlings and House Sparrows in Great Britain. (Crick, H., Robinson, R., Appleton, G., Clark, N.A. and Rickard, A.). BTO Report Number 290 (BTO/DEFRA).
- Crow JF, Aiko K (1984). Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *PNAS* 19: 6073–6077.
- Dawson DA, Horsburgh GJ, Krupa A, Stewart IRK, Skjelseth S, Jensen H, et al. (in review). A predicted microsatellite map of the house sparrow *Passer domesticus* genome. (*Re-submitted to Mol Ecol Res*).
- De Laet J, Summers-Smith JD (2007). The status of the urban house sparrow *Passer domesticus* in north-western Europe: a review. *Journal of Ornithology* 148 (Suppl. 2): 275–278.
- DeSalle R, Amato G (2004). The expansion of conservation genetics. *Nature reviews genetics*. 5: 702–712.
- Eeva T, Hakkarainen H, Belskii E (2009). Local survival of pied flycatcher males and females in a pollution gradient of a Cu-smelter. *Environmental Pollution* 157: 1857–1861.
- Ellegren H (2000). Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics*. 16: 551–558.
- Evanno G, Castella E, Goudet J (2006). Evolutionary aspects of population structure for molecular and quantitative traits in the freshwater snail *Radix balthica*. *Journal of Evolutionary Biology* 19: 1071–1082.
- Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47–50.
- Falush D, Stephens M, Pritchard JK (2003). Inference of population structure: Extensions to linked loci and

- correlated allele frequencies. *Genetics* 164: 1567–1587.
- Falush D, Stephens M, Pritchard JK (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes* 7: 574–578.
- Frankel OH, Soulé ME (1981). *Conservation and evolution*. Cambridge University Press, Cambridge, UK.
- Frankham R (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10: 1500–1508.
- Frankham R (1999). Quantitative genetics and conservation biology. *Genetical research*. 74 (3): 237–244.
- Frankham R, Ballou J, Briscoe D (2002). *Introduction to conservation genetics*. 1st edition, Cambridge University Press.
- Frankham R, Ballou J, Briscoe D (2009). *Introduction to conservation genetics*. 2nd edition, Cambridge University Press.
- Franklin IR (1980). *Evolutionary changes in small populations*. In: Conservation Biology (eds Soule & Wilcox), 135–149. Sinauer.
- Glenn TC, Stephan W, Braun MJ (1999). Effects of a Population Bottleneck on Whooping Crane Mitochondrial DNA Variation. *Conservation Biology* 13 (5): 1097–1107.
- Goldstein DB, Schlötterer C (1999). *Microsatellites, Evolution and Applications*. Oxford University Press.
- Goudet J (2001). *FSTAT: A Program to Estimate and Test Gene Diversities and Fixation Indices*, Version 2.9.3. Lausanne University, Lausanne, Switzerland.
- Goudet J (2005). HIERFSTAT, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes* 5:184–186.
- Goyal M. (2005). M. Sc. Dissertation: Preliminary Survey of House sparrow (*Passer domesticus*) in three Different Areas of Haridwar, Uttaranchal. — Wildlife Institute of India, Dehradun. Gurukul Kangri University, Haridwar.
- Griffith SC, Stewart IRK, Dawson DA, Owens IPF, Burke T (1999). Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an “island effect”? *Biological Journal of the Linnean Society* 68: 303–316.
- Griffith SC, Dawson DA, Jensen H, Ockendon N, Greig C, Neumann K, et al. (2007). Fourteen polymorphic microsatellite loci characterized in the house sparrows *Passer domesticus* (Passeridae, Aves). *Molecular Ecology Notes* 7: 333–336.
- Groombridge JJ, Jones CG, Bruford MW, Nichols RA (2000). Conservation biology: “Ghost” alleles of the Mauritius kestrel. *Nature* 403: 616.
- Hadfield J (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software* 33(2): 1–22.
- Hartl DL, Clark AG (2007). *Principles of Population Genetics*, 4th edn, Sinauer Associates.
- Hitt, N.P. et al. (2003) Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences* 60:1440–1451.
- Holand AM, Jensen H, Tufto J, Moe R (in review). Does selection or genetic drift explain geographic differentiation of morphological characters in house sparrows *Passer domesticus*?
- Hole DG, Whittingham MJ, Bradbury RB, Anderson GQA, Lee PLM, Wilson DJ, Krebs JR (2002). Widespread local house sparrow extinctions. *Nature* 418: 139–149.
- Hutchinson WF, van Oosterhout C, Rogers SI, Carvalho GR (2003). Temporal analysis of archived samples indicates genetic changes in declining North Sea cod (*Gadus morhua*). *Proceedings of the Royal Society London B*. 270:2125–2132.
- Janssens E, Dauwe T, Pinxten R, Bervoets L, Blust B, Eens M (2003). Effects of heavy metal exposure on the condition and health of nestlings of the great tit (*Parus major*), a small songbird species. *Environmental Pollution* 126: 267–274.
- Jensen H, Moe R, Holland AM, Tufto J, Sæther B-E (In prep.) Genetic variation and structure of house sparrow populations: is there an island effect?
- Jensen H, Sæther BE, Ringsby TH, Tufto J, Griffith SC, Ellegren H (2003). Sexual variation in heritability and genetic correlations in morphological traits in house sparrow (*Passer domesticus*). *Journal of Evolutionary Biology* 16: 1296–1307.
- Jensen H, Steinsland I, Ringsby TH, Sæther BE (2008). Evolutionary dynamics of a sexual ornament in the house sparrow (*Passer domesticus*): the role of indirect selection within and between sexes. *Evolution* 62: 1275–1293.
- Kalinowski ST (2005). HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes* 5: 187–189.
- Koskinen MT, Sundell P, Piironen J, Primmer CR (2002). Genetic assessment of spatiotemporal evolutionary relationships and stocking effects in grayling (*Thymallus thymallus*, Salmonidae). *Ecological Letters* 5:193–205.
- Kruszewicz AG, Kruszewicz AH, Pawiak R, Mazurkiewicz M (1995). The International Symposium of the Working Group on Granivorous Birds – Granivorous Birds as Agricultural Pests and Epidemiological Vectors. *Bacteria in House Sparrow (Passer domesticus) and Tree Sparrow (Passer montanus) nestlings: occurrence and influence on growth and mortality*. — In Nestling mortality of granivorous birds due to microorganisms and toxic substances: Synthesis, (Eds. Pinowski J, Kavanagh B and Pinowska B).
- Lee CE, Frost BW (2002). Morphological stasis in the *Eurythemora affinis* species complex (Copepoda: Temoridae). *Hydrobiologia* 480: 111–128.
- Lehtonen P, Laaksonen T, Artemyev AV, Belskii E, Both

- C, Bures S, Bushuev AV, Krams I, Moreno J, Magi M, Nord A, Potti J, Ravussin PA, Sirkia PM, Saetre GP, Primmer CR (2010). Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Molecular Ecology* 21: 4463–4476.
- Leinonen T, Cano JM, Makinen H, Merilä J (2006). Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology* 19: 1803–1812.
- Leinonen T, O'Hara R, Cano JM, Merilä J (2008). Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *Journal of Evolutionary Biology* 21: 1–17.
- Leonard JA (2008). Ancient DNA applications for wildlife conservation. *Molecular Ecology* 17: 4186–4196.
- Lowe A, Harris S, Ashton P (2004). *Ecological Genetics: Design, Analysis and Application*, 1st edn. Blackwell Publishing: Oxford.
- Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998). Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology* 7:963–974.
- Luikart, G, Cornuet J-M, Allendorf FW (1999). Temporal changes in allele frequencies provide estimates of population bottleneck size. *Conservation Biology* 13:523–530.
- Martinez-Cruz B, Godoy JA, Negro JJ (2007). Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. *Molecular Ecology* 16: 477–486.
- Matocq MD, Villablanca FX (2001). Low genetic diversity in an endangered species: recent or historic pattern? *Biological Conservation* 98: 61–68.
- Maynard Smith J (2000). *Evolutionary Genetics*. 2nd Oxford University Press.
- McCarthy M (2003). Wood Pigeons' grain diet linked to the demise of House Sparrows. The Independent. 3rd September 2003.
- McKay JK, Latta, RG (2002). Adaptive population divergence: Markers, QTL and traits. *Trends Ecology and Evolution* 17: 285–291.
- McKelvey KS, von Kienast J, Aubry KB, Koehler GM, Maletzke BT, Squires JR, Lindquist EL, Loch S, Schwartz MK (2006). DNA analysis of hair and scat collected along snow tracks to document the presence of Canada lynx (*Lynx canadensis*). *Wildlife Society Bulletin* 34: 451–455.
- Merilä J, Crnokrak P (2001). Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14: 892–903.
- Mikkola-Roos M, Tiainen J, Below A, Hario M, Lehikoinen A, Lehikoinen E, Lehtiniemi T, Rajasärkkä A, Valkama J, Väisänen RA (2010). Birds. In: Rassi P, Hyvärinen E, Juslén A, Mannerkoski I (eds. 2010). *The 2010 Red List of Finnish Species* Ministry of the Environment and Finnish Environment Institute, Helsinki.
- Miller JR, Hamilton MB, Wood BP (2008). F_{ST} and Q_{ST} under neutrality. *Genetics* 180: 1023–1037.
- Munoz-Fuentes V, Green AJ, Negro JJ, Sorenson MD (2005). Population structure and loss of genetic diversity in the endangered white-headed duck, *Oxyura leucocephala*. *Conservation Genetics* 6: 999–1015.
- Nei M (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Neumann K, Wetton K (1996). Highly polymorphic microsatellites in the house sparrow *Passer domesticus*. *Molecular Ecology* 5: 307–309.
- Palm S, Laikre L, Jorde PE, Ryman N (2003). Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta*, L.). *Conservation Genetics* 4: 249–264.
- Palo JU, O'Hara RB, Laugen AT, Laurila A, Primmer CR, Merilä J (2003). Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. *Molecular Ecology* 12: 1963–1978.
- Payne RB, Sorensen MD (2002). Museum collections as sources of genetic data. *Bonner Zoologische Beiträge Band* 51: 97–104.
- Peach WJ, Vincent KE, Fowler JA, Grice PV (2008). Reproductive success of house sparrows along an urban gradient. *Animal Conservation* 11: 493–503.
- Peterjohn BG, Sauer JR, Link WA (1994). The 1992 and 1993 summary of the North American Breeding Bird Survey. *Bird Populations* 2: 46–61.
- Pichler FB, Baker CS (2000). Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality. *Proceedings of the Royal Society B* 267(1438): 97–102.
- Piggott, MP, Banks SC, Stone N, Banffy C, Taylor AC (2006). Estimating population size of endangered brush-tailed rock-wallaby (*Petrogale penicillata*) colonies using faecal DNA. *Molecular Ecology* 15: 81–91.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.
- Pullin AS (2002). *Conservation biology*. Cambridge University Press
- Rassi P (2000). Lintujemme uusi uhanalaisuustarkastelu. *Linnut* 2/2000 (Linnut-magazine, in Finnish).
- Rassi P, Alanen A, Kanerva T, Mannerkoski I (2001). *Suomen lajien uhanalaisuus 2000*. — Ympäristöministeriö & Suomen ympäristökeskus, Helsinki.
- Reed DH, Frankham R (2001). Correlation between Fitness and Genetic Diversity. *Conservation Biology* 17: 230–237.
- Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J, Burke T (2000). Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species

- amplification in other passerine birds. *Molecular Ecology* 9: 2155–2234.
- Rudnick JA, Katzner TE; Bragin EA; Rhodes OE JR, De-Woody JA (2005). Using naturally shed feathers for individual identification, genetic parentage analysis, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Molecular Ecology* 14: 2959–2967.
- Sæther SA, Fiske P, Kålås JA, Kuresoo A, Luigujoe L, Pi-ertney SB, Sahlman T, Höglund J (2007). Inferring local adaptation from QST-FST comparisons: neutral genetic and quantitative trait variation in European populations of Great Snipe. *Journal of Evolutionary Biology* 20: 1563–1576.
- Schwartz MK, Luikart G, Waples RS (2007). Genetic monitoring as a genetic tool for conservation and management. *Trends in Ecology and Evolution* 22:25–33.
- Siriwardena G, Robinson R, Crick H (2002). *Status and population trends of the House Sparrow Passer domesticus in Great Britain*. Investigation into the causes of the decline of Starlings and House Sparrows in Great Britain. (Crick, H., Robinson, R., Appleton, G., Clark, N. and Rickard, A.). BTO Report Number 290. (BTO/DEFRA).
- Skjelseth S, Ringsby TH, Tufto J, Jensen H, Sæther BE (2007). Dispersal of introduced house sparrows *Passer domesticus* – an experiment. *Proceedings of the Royal Society B* 274: 1763–1771.
- Soulé ME (1987). *Viable populations for conservation*. Cambridge University Press.
- Spencer CC, Negel JE, Leberg PL (2000). Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. *Molecular Ecology* 9: 1517–1528.
- Spielman D, Brook BW, Frankham R (2004). *Proceedings of the National Academy of Science USA* 101: 15261–15264.
- Spitze, K (1993). Population structure in *Daphnia obtusa* – quantitative genetic and allozymic variation. *Genetics* 135: 367–374.
- Summers-Smith JD (1988). *The Sparrows*. T. & A.D. Poyser: Staffordshire.
- Summers-Smith JD (1999). Current status of the house sparrow in Great Britain. *British Wildlife* 10:381–386.
- Summers-Smith JD (2003). Decline of the House sparrow: a Review. — British Birds, www.ndoc.org.uk.
- Summers-Smith JD (2006). *On Sparrows and Man: A Love-hate Relationship*. Private publish of JD Summers-Smith.
- Swaileh KM, Sansur R (2006). Monitoring urban heavy metal pollution using the House Sparrow (*Passer domesticus*). *Journal of Environmental Monitoring* 8: 209–213.
- Schwartz MK, Luikart G, and Waples RS (2007). Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution* 22: 25–33.
- Szulkin M, Bierne N, David P (2010). Heterozygosity-Fitness Correlations: A time for reappraisal. *Evolution* 64:1202–1214.
- Tufto J, Ringsby TH, Dhondt AA, Adriaensen F, Matthysen E (2005). A parametric model for estimation of dispersal patterns applied to five passerine spatially structured populations. *American Naturalist* 165:13–26.
- Varvio SL, Chakraborty R, Nei M (1986). Genetic variation in subdivided populations and conservation genetics. *Heredity* 57: 189–198.
- Vincent, K. (2005). Ph. D. thesis: Investigating the causes of the decline of the urban House Sparrow *Passer domesticus* population in Britain. — De Montfort University, Great Britain.
- Väisänen RA, Lammi E, Koskimies P (1998). *Muuttuva pesimälinnusto*. — Otava, Keuruu. (in Finnish with English summary).
- Väisänen RA (2003). Regional population trends of 33 common bird species in Finland during 27 winters. *The Yearbook of the Linné Magazine* 2002: 41–62.
- Wandeler P, Hoeck PEA, Keller LF (2007). Back to the future: museum specimens in population genetics. *Trends Ecology and Evolution* 22: 634–642.
- Wang J (2005). Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society B* 360:1395–1409.
- Weir B, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.
- Whitlock MC (2008). Evolutionary inference from Q_{ST} . *Molecular Ecology* 17 (8):1885–1889.
- Wright S (1943). Isolation by distance. *Genetics* 28: 114–137.
- Yom-Tov Y (2001). Global warming and body mass decline in Israeli passerine birds. *Proceedings of the Royal Society of London B* 268: 947–952.