Genetic differentiation within and between bird populations – taxonomic and phylogeographic implications

by

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Abstract

The mapping of genealogical relationships between individuals, populations, subspecies and species is important for studies of evolutionary processes and biodiversity, and for conservation decisions. In this thesis, I have used several different types of molecular markers to investigate neutral genetic variation and structure at the population, subspecies and species level in three bird species or species complexes distributed in arctic and temperate regions; snowy owl (*Bubo scandiacus*), dunlin (*Calidris alpina*) and redpoll (*Carduelis flammea*, *C. cabaret*, *C. hornemanni*). In addition to providing knowledge on evolutionary processes shaping genetic patterns, the analyses have implications for conservational decisions for the studied species living in the arctic environment where climatic changes may have severe impacts. I found low levels of genetic structure in all investigated groups. Snowy owls from three well separated geographic regions were analyzed with two mtDNA genes and two Z-chromosome introns and they seemed to constitute one panmictic population. The species was also relatively genetically diverse compared to three other owl species breeding in temperate regions. The pattern found with four genetic markers (mtDNA control region, a Z-chromosome intron, microsatellites and AFLPs) among dunlin populations in Western Palearctic and East Greenland did not correspond to the four subspecies recognized in the area. Rather, dunlins in this area form two genetically monophyletic groups that are geographically overlapping, and the resulting pattern is isolation by distance. Declining and fragmented dunlin populations of the subspecies southern dunlin (*C. a. schinzii*) were not genetically deprived compared to vital populations in more continuous habitats. Nor was the isolated dunlin population on Svalbard, this population was genetically similar to populations on East Greenland, Iceland and around the Baltic Sea. The three redpoll species were exceptionally little differentiated in mtDNA control region sequences and microsatellites. The low level of genetic structure in dunlins and redpolls is surprising considering their taxonomic status. Recent divergence of morphological characters or ongoing gene flow may explain the patterns found in both species. I suggest that the clinal variation described illustrates a more common pattern than is normally acknowledged in avian systematics, and that conservation politics may benefit from taking these patterns into consideration instead of being constrained to categorical thinking. This is important in today's situation of declining population sizes and threatened species in an increasing number of areas and habitats.

Introduction

Taxonomy – the theory and practice related to the classification of organisms – is the basis for studies of patterns and processes in nature, as well as for conservation decisions. Classification of organisms is however not always a straightforward task, as illustrated for example by the long-lasting and emotional debate about species concepts (Wheeler and Meier 2000). Some species concepts define species based on reproductive barriers (the Biological Species Concept, Mayr 2000, and the Hennigian Species Concept, Meier and Willmann 2000), some on unique combinations of character states (*e.g.* the Phylogenetic Species Concept *sensu* Wheeler and Platnick 2000), and some on monophyly or evolutionary fate (*e.g.* the Phylogenetic Species Concept *sensu* Mishler and Theriot 2000, and the Evolutionary Species Concept, Wiley and Mayden 2000, respectively). Also classifications below the species level can be based on different criteria. A comprehensive definition of a subspecies is a group of populations within a species that share a unique geographic range or habitat, and differ from other such groups in several genetically based traits, *e.g.* morphology and ecology (Avise and Ball 1990, Ball and Avise 1992, Frankham et al. 2004). Other definitions focus more on patterns in neutral genetic markers because of their exposure of evolutionary history (Zink 2004). Regarding conservation, there has been a debate on whether adaptive evolution affecting fitness or long term historical isolation is most important, *i.e.* phenotypic variation or molecular phylogeography (Crandall et al. 2000, Moritz 2002). Phylogeography is the geographic mapping of neutral genetic structure within species, and the resulting pattern may reveal major historical lineages and historic changes in population sizes and ranges (Avise et al. 1987, Avise 2000).

As long as phenotypic and neutral genetic variation correspond, taxonomical decisions are relatively straightforward. However, sometimes there is a lack of correspondence between structure in neutral genetic markers and morphological characters (*e.g.* Bensch et al. 1999, Haavie et al. 2000, de Knijff et al. 2001). Some studies have also revealed differing patterns in different neutral genetic markers (*e.g.* Waits et al. 2000, Irwin et al. 2001, Crochet et al. 2003, Johnson et al. 2003, Sønstebø et al. 2007). Such discrepancies occur because the factors shaping structure; gene flow, genetic drift and natural and sexual selection, act differently, or are of varying relative importance in different habitats and regions, and in different marker types (Avise 2000, Hedrick 2001).

The neutral genetic patterns of species breeding in arctic and temperate regions have to a great extent been formed during the Pleistocene ice ages (Ploeger 1968, Avise and Walker 1998, Avise et al. 1998, Klicka and Zink 1999). During this period, which lasted from about two million years to 10 000 years ago, several glaciation events, each spanning ca 100 000 years, were interrupted by interglacial periods lasting 10 000- 12 000 years (Martinson et al. 1987, Dawson 1992). Within isolated glacial refugia, genetic drift and novel mutations created differences between refugial populations (Hewitt 2004). After the melting of the ice, the formerly glaciated areas were recolonized, and the refugial populations met. In cases where the groups had not diverged too much, the diverged genomes hybridized (Hewitt 2001), while in other cases they had formed reproductively isolated groups.

In my thesis I have investigated neutral genetic diversity and structure using several genetic markers in three taxonomic groups of birds living in arctic and temperate regions. In the Arctic, climatic changes may have particularly profound effects (Chapin et al. 2006), and may consequently be the most important area to gain knowledge on genetic patterns for conservation decisions and future evolutionary changes in. The investigated species – snowy owl, dunlin and redpoll – were chosen because of their various ecology, behaviour, and taxonomic resolution. The snowy owl is not divided into subspecies and was investigated at the population level. The dunlin consists of up to 11 subspecies and was investigated at the population and subspecies level. Redpolls are divided into three closely related species and were investigated at the subspecies and species level. The genetic patterns will be discussed in relation to the groups' taxonomy, their ecology and behaviour, and their management status.

Study species and questions

Snowy owl (*Bubo scandiacus*)

The snowy owl (Fig. 1) has a circumpolar distribution and breeds in the Arctic (Cramp and Perrins 1994, Fig. 2). It preys on rodents occurring in highly variable densities, mostly lemmings (*Lemmus* and *Dicrostonyx* spp.) and voles (*Microtus* and *Clethrionomys* spp.), and is particularly dependent on high densities when breeding. Consequently, snowy owls need to move where the food is abundant, and are thus fluctuating in numbers in different

areas (Alerstam 1990, Cramp and Simmons 1994). The snowy owl's capacity of long distance movements was investigated with satellite telemetry by Fuller et al. (2003). Owls were found to travel distances more than 3000 km in a few weeks. It is however not known whether they form one panmictic population across their entire distribution, or if there are barriers to gene flow. No phylogeographic studies have been performed for snowy owls.

Figure 1 Snowy owl *Bubo scandiacus*. Photo: Frode Jacobsen

The number of snowy owls in the world has been estimated to be 290 000 and is assumingly stable (BirdLife International 2004). However, in Western Palearctic, snowy owls have been reported to decline in numbers during the last century (Portenko 1972, Solheim 1994), and it has status as Vulnerable, Endangered and Critically Endangered in the red lists of Norway, Finland and Sweden, respectively (Rassi et al. 2001, Gärdenfors 2005, Kålås et al. 2006).

The main question I have asked is whether snowy owls constitute one panmictic population or whether there are barriers to gene flow (Paper I). I analyzed snowy owls from three geographically separated regions with several genetic markers to see if there was any genetic structure. I also analyzed genetic diversity within the regions to see if the

birds in the Western Palearctic were less diverse compared to the regions where they have not been reported to decline. To asses the species-level genetic diversity of snowy owl, I analyzed Scandinavian populations of the three owl species eagle owl (*Bubo bubo*), tawny owl (*Strix aluco*) and Tengmalm's owl (*Aegolius funereus*), all breeding in temperate regions.

Figure 2 Breeding distribution of snowy owls *Bubo scandiacus*, modified from Cramp and Simmons (1994).

Dunlin (*Calidris alpina*)

The dunlin (Fig. 3) is a circumpolarly distributed wader breeding in temperate and arctic regions (Cramp and Simmons 1983, Fig. 4). There is considerable morphological and genetic variation within the species. Up to 11 subspecies have been described based on plumage, body size, bill length, migration routes and moulting pattern (Cramp and Simmons 1983, Greenwood 1986, Hayman et al. 1986, del Hoyo et al. 1996, Engelmoer and Roselaar 1998). Five of these subspecies are described from Western Palearctic and the eastern coast of Greenland. The nominate subspecies *C. a. alpina* (Linneaus 1758) breeds in the mountain areas of Scandinavia and eastwards along the Russian coast (Cramp and Simmons 1983, Fig. 4). At Taimyr Peninsula, *alpina* borders *C. a. centralis* (Buturlin 1932), which is found east to Kolyma River (Vaurie 1965, Fig. 4). The *centralis* subspecies is not generally recognized by most handbooks (*e.g.* Cramp and Simmons 1983, del Hoyo et al. 1996), but differs from *alpina* both in morphology, moult phenology and migration pattern (Buturlin 1932, Greenwood 1983, Gromadzka 1989, Engelmoer and Roselaar 1998, Holmgren et al. 2001). In southern Norway, *alpina* borders *C. a. schinzii* (Brehm 1822). In addition to coastal areas in southern Norway, *schinzii* breeds around the Baltic Sea, on the coasts of western Sweden, Denmark and Germany, in the Wadden See, on the British Isles and on Iceland (Cramp and Simmons 1983, Fig. 4). The Icelandic dunlins were described as a separate subspecies *C. a. islandica* by Schiøler (1922) based on measures of Icelandic museum skins being intermediate between *alpina* and *schinzii* in size and colour. They are considered to belong to *schinzii* today (Cramp and Simmons 1983). The *schinzii* subspecies differs from *alpina* in plumage (von Blotzheim et al. 1975), bill length, body size (Engelmoer and Roselaar 1998), migration pattern (Cramp and Simmons 1983) and breeding habitat (Emanuelsson and Kjellén 1981). Dunlins breeding on the north-east coast of Greenland constitute the subspecies *C. a. arctica* (Schiøler 1922). These birds are smaller, have shorter bills and differ in plumage from *alpina* and *schinzii* (Schiøler 1922, Engelmoer and Roselaar 1998).

The dunlin subspecies in Western Palearctic and East Greenland are thus delimitated based on morphology as well as behavioural and ecological characters and they mostly occupy non-overlapping geographic areas. The exception is southern Norway where *alpina* and *schinzii* meets; there is no consensus about where the border is. The populations on Hardangervidda, and on the coasts of Møre and Trøndelag in Norway are reported to be intermediate between *alpina* and *schinzii* in biometric measures

Figure 3 Dunlin *Calidris alpina* on Hitra, Norway. Photo: Gunnhild Marthinsen

(Kålås and Byrkjedal 1981, Fiske 1994), and I have in my studies regarded the region as a hybrid zone (Fig. 4).

Genetically, dunlins worldwide constitute five mtDNA clades (Wenink et al. 1993, Wenink et al. 1996, Wennerberg et al. 1999, Wennerberg 2001). Two of these are found in Western Palearctic and East Greenland; the European clade EUR and the Siberian clade SIB (Wenink et al. 1993, Wenink et al. 1996, Wennerberg et al. 1999, Wennerberg 2001, Lopes et al. 2006). EUR is found on Greenland, Iceland, the British Isles, around the Baltic Sea, in Scandinavia and in Russia east to Taimyr. SIB is distributed mainly in Siberia, but overlaps with the EUR clade and is found in small frequencies as far south as Hardangervidda (Wenink et al. 1996). The two clades were probably differentiated in different Pleistocene refugia (Wenink et al. 1996), and have since met and mixed.

Dunlin populations breeding around the Baltic Sea, along the coast in southern Norway and along the Kattegat coast have declined in size during the last 50 years due to human destructions of habitat and changes in agricultural management (Larsson 1969,

Soikkeli and Salo 1979, Emanuelsson and Kjellén 1981, Perttula 1990, Renno 1994, Wlodarczak 1999, Thorup 2004, Breiehagen 2006).

In three papers I have investigated the genetic structure among dunlin populations in Western Palearctic and East Greenland using four different genetic markers. I aimed to investigate the molecular support for the subspecies taxonomy and maybe infer conservation units below the subspecies level. In the first dunlin paper (Paper II) I analyzed populations from across the region using two genetic marker types

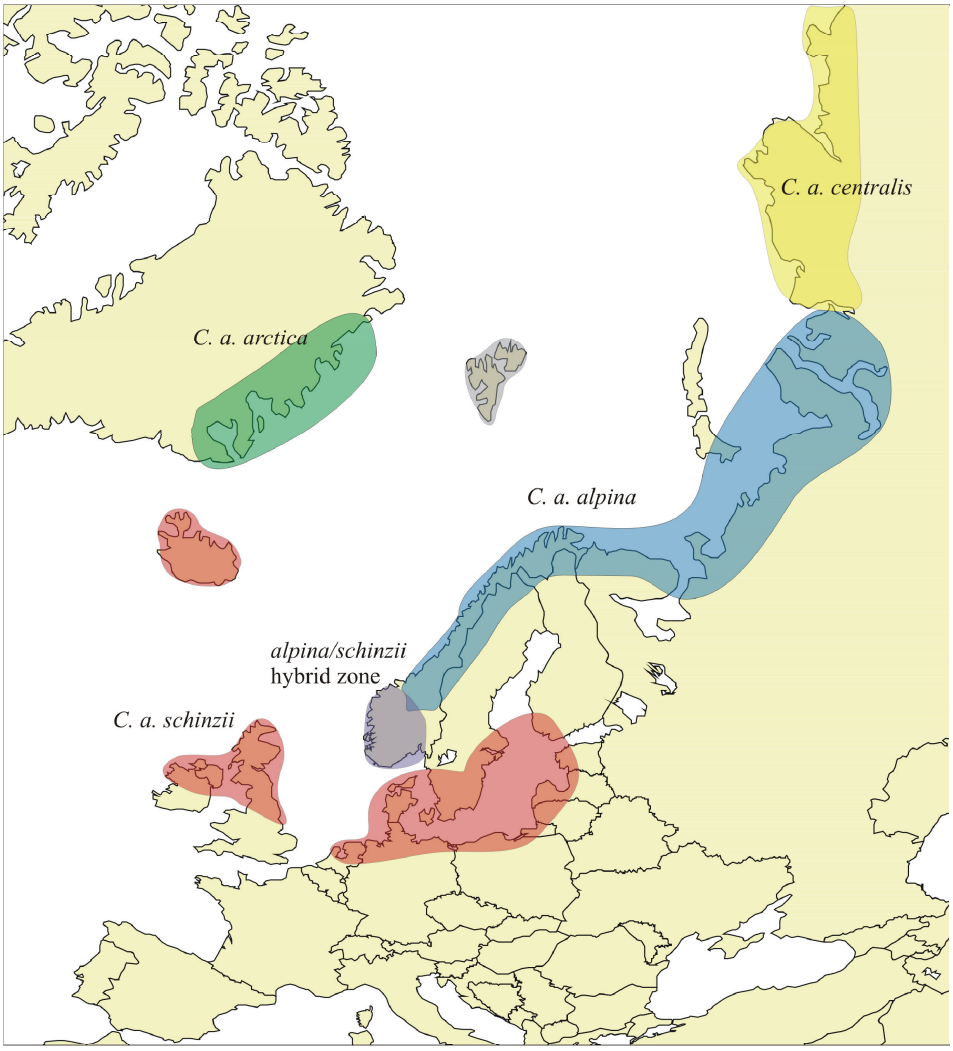

Figure 4 Distribution of dunlin subspecies in Western Palearctic and Greenland, following Cramp and Simmons (1983).

(microsatellites and AFLPs) that could potentially give a higher resolution among populations than the above described mtDNA control region. In the second dunlin paper (Paper III) I analyzed populations from the subspecies *alpina*, *schinzii* and *arctica* with all four genetic marker types described below. The main question here was whether the *schinzii* subspecies was genetically distinct, and whether there was a structure within the subspecies that separated the threatened populations around the Baltic Sea from the apparently non-threatened population in Iceland. I also investigated within-population diversity to see if the threatened populations were less diverse than the Iceland population and non-threatened populations of *alpina*. The third dunlin paper (Paper IV) concerns the dunlin population in Svalbard, whether this small and isolated population is genetically deprived or inbred, and what region in Western Palearctic it resembles most genetically.

Redpolls (*Carduelis flammea – hornemanni – cabaret*)

Redpolls (Fig. 5) eat seeds, particularly from birch and spruce, and thus have an irruptive dispersal behaviour; they occur in large numbers in areas where these seeds are abundant (Troy 1983). They are opportunistic breeders and have low local return rates (Troy 1983).

Two redpoll species are distributed circumpolarly; common redpoll (*C. flammea*) and Arctic redpoll (*C. hornemanni*). Common redpoll breeds mainly below the tree line and Arctic redpoll breeds at the tundra and at the forest edges (Knox 1988, Fig. 6). The redpoll complex consists of one more species in addition to these two; the lesser redpoll (*C. cabaret*). This race was previously a subspecies of the common redpoll, but was recently given species status (Knox et al. 2001, Sangster et al. 2002). Common redpoll is divided into the subspecies mealy redpoll (*C. f. flammea*) breeding in northern Eurasia and North America (except Baffin Island), greater redpoll (*C. f. rostrata*) breeding on Baffin Island and southern Greenland, and Iceland redpoll (*C. f. islandica*) breeding on Iceland (Knox 1988, Fig. 6). Arctic redpoll is divided into hoary redpoll (*C. h. exilipes*) found in northern Eurasia and North America, and Greenland redpoll (*C. h. hornemanni*) found on Ellesmere Island, Baffin Island and northern Greenland (Fig. 6). The taxonomy of the group has been discussed thoroughly (summary in Knox 1988), and the complex has been termed a taxonomic enigma (Dawson and Allsopp 1985). This is due to an extensive degree of morphological variation, particularly in biometric measures and plumage patterns, within the complex (reviewed in Knox 1988), and there has been opposing views

on whether there are one, two or three species (Harris et al. 1965, Molau 1985, Troy 1985, Knox 1988, Seutin et al. 1992). Genetic studies have revealed a low resolution also at the molecular level; RFLP analyses have shown no differences between any investigated species or subspecies (Marten and Johnson 1986, Seutin et al. 1995), and Ottvall et al. (2002) found no differences between *C. flammea* and *C. cabaret* in mtDNA control region sequences.

Figure 5 Mealy redpoll *Carduelis flammea flammea*. Photo: Lars Erik Johannessen

The redpoll species' low degree of differentiation indicates that they may be recently diverged species, and knowledge on their genetic patterns may give insights into speciation processes. In addition, although redpolls are not reported to be threatened in any region, future climatic changes in their arctic habitat may call for management decisions also in this group.

I have extended on the above mentioned genetic studies on redpolls by analyzing highly mutable microsatellites, and adding mtDNA control region sequences for the *hornemanni* species and the *flammea rostrata* subspecies and more breeding populations of

the *flammea* and *cabaret* species (Paper V). The *flammea* and *cabaret* samples analyzed by Ottvall et al. (2002) were all sampled in Scandinavia, either in breeding populations or on migration, and the inclusion of other breeding populations and additional taxa may reveal differences not yet detected. Microsatellites mutate faster than the mtDNA control region and could therefore give a higher resolution (Hewitt 2001). As an outgroup I used the twite (*C. flavirostris*) including two subspecies; *flavirostris* and *rufostrigata*, sampled in Norway and Tibet, China, respectively.

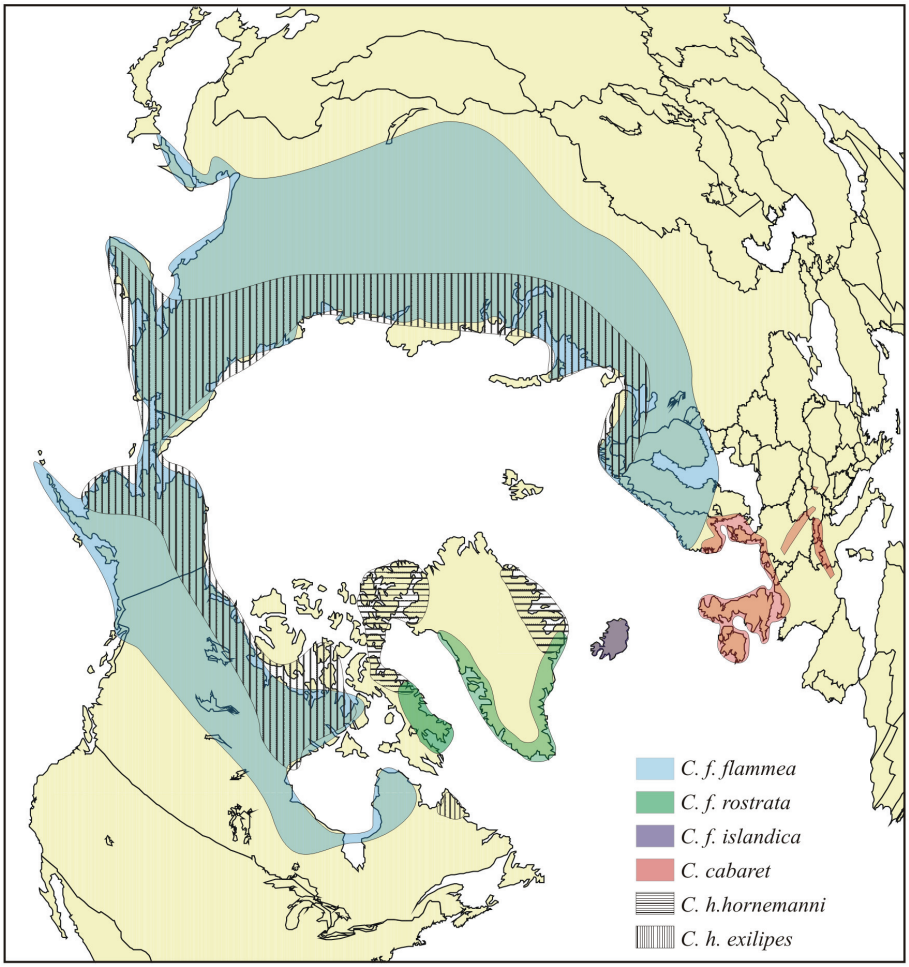

Figure 6 Distribution of redpoll species and subspecies, following Knox et al. (1988).

Molecular markers

MtDNA has been extensively used in phylogeographic studies, partly because of its serendipity (Avise 1998, Hewitt 2001), which means "an aptitude for making desirable discoveries by accident". More scientifically, mtDNA is a good genetic marker for investigating genetic relationships at and below the species level because of its high mutation rate (which may vary greatly among regions, Hewitt 2001), and because of its inheritance pattern and small effective population size. MtDNA is maternally inherited, and thus the tracing of mtDNA genes between generations of ancestors and descendants is a possible task. Furthermore, the effective population size (which reflects the actual number of individuals that contribute genetically to the next generation) of this genome is ¼ of the nuclear autosomal genome (Avise 2000). Thus, the coalescence time (time since last common ancestor) of mtDNA is also ¼ that of nuclear sequences, and reciprocal monophyly is reached faster for separated populations (Avise 2000).

I have sequenced the control region for all studied taxa. This part of the mtDNA molecule has a relatively high mutation rate in most species, although it varies to a great extent; *e.g.* 4.54% per Million year in grouse (*Tetraoninae*, Drovetski 2003), 14.8% in dunlins (Wenink et al. 1996) and 20.8% in lesser snow goose (*Chen caerulescens*, Quinn 1992). The region does not code for any proteins, but is associated with replication (Avise 2000). For snowy owls, I also sequenced cytochrome *b* (Cyt *b*), which is a coding gene on the mtDNA, with an assumed mutation rate of 2% per Million year (Hewitt 2001). I also attempted to sequence the NADH dehydrogenase subunit II for snowy owls, but for most individuals two fragments were amplified in the first PCR. Because of time constraint I did not go further in optimizing the PCR conditions or primers.

Nuclear autosomal loci are difficult to sequence because all individuals have two alleles (one from mother and one from father). One way to get single nuclear alleles without molecular cloning is to target sex-chromosome loci in the heterochromatic sex; *i.e.* Z-chromosome loci or W-chromosome loci in females for birds (females have one Z- and one W-chromosome; males have two Z-chromosomes). I have sequenced introns found on the Z-chromosome (VLDLR-9 and BRM15 for snowy owls in paper I, and VLDLR-9 for dunlins, paper III), for both males and females (if using only females I would have got too low sample sizes). For snowy owls, there was only one variable base for both markers, and males could therefore also be used, and for dunlins, a large proportion of the sequenced

fragment could be used for both sexes. Indels in some haplotypes allowed for entangling of the two haplotypes in males with two different alleles. This way I may have missed some variation, but this is a conservative approach. I also sequenced some snowy owl individuals for the Z-chromosome intron VLDLR-7, but because there was no variation in a subset of samples, I did not prioritize further analyses.

Other ways to get information on nuclear DNA is fragment or fingerprinting analyses. I have analyzed microsatellites and AFLP.

Microsatellites are sequences made up of tandemly repeated short sequence motifs of 2-6 base pairs (Goldstein and Schlötterer 1999). The repeated motifs lead to high rates of slipped strand mispairing (slippage) during DNA replication (Goldstein and Schlötterer 1999). Consequently, microsatellites have very high mutation rates; 10^{-3}-10^{-6} mutations per locus per generation (Hewitt 2001), so potentially, high levels of variation may be created in relatively short time. The mutation mode for microsatellites is widely discussed, and several models have been suggested. In short, the infinite allele model (IAM, Kimura and Crow 1964) assumes that each mutation creates a new allele by adding or subtracting any number of tandem repeats. The stepwise mutation model (SMM, Kimura and Ohta 1978) predicts that one or a few repeats are added or subtracted in each mutation event. The two-phase model (TPM, Di Rienzo et al. 1994) allows loss and gain of several repeats following a geometric distribution in each mutation. There is no unequivocal answer to which model is best suited for microsatellites (van Oppen et al. 2000). Different microsatellite loci probably have different evolutionary dynamics because of factors like repeat number, sequence of the repeat motif, length of the repeat unit, flanking sequence, interruptions in the microsatellite, recombination rate, transcription rate, age and sex, and efficiency of the mismatch repair system (Estoup et al. 2002). Also, not all mutations related to microsatellites correspond to stepwise mutations. Mutations involving length alterations of a number of base pairs not corresponding to the repeat motif may happen in the flanking region, or within the microsatellite, leading to alleles differing by imperfect repeats (Khasa et al. 2000, Hughes et al. 2002). Mutations in the flanking region have actually nothing to do with the microsatellite, but fragment analyses cannot separate these from mutations within the actual microsatellite. In my microsatellite studies, 10 of 17 microsatellite loci involved alleles differing from the other alleles by a lower number of base pairs than the repeat length (Paper I and V).

I have used the TPM model in some tests because this has been said to be the most realistic mutation model for microsatellites (Di Rienzo et al. 1994, Estoup and Cornuet

1999). In other tests, where the TPM model was not available, I have used the IAM model because of the imperfect length differences in several loci not compatible with the other mutation models. In tests where several mutation models were available I tried all and revealed insignificant differences between the obtained results.

The other fingerprinting method I have used is amplified fragment length polymorphism (AFLP). In this method, the DNA is cut with restriction enzymes, and a subset of the resulting fragments in the range 100-500 bp are amplified (Mueller and Wolfenbarger 1999). With this method, the whole genome is screened in an efficient way, potentially revealing a lot of variation. There are however some disadvantages with the AFLP method. The amplified fragments are for example non-specific, in the sense that we do not know on what part of the genome they are situated, or whether they are coding or neutral (Vos et al. 1995). Furthermore, the resulting markers from an AFLP analysis are dominant, meaning that heterozygotes cannot be distinguished from dominant homozygotes. This makes the estimation of allele frequencies difficult (Lynch and Milligan 1994). The scoring of AFLP fragments may also be quite cumbersome, and high error rates may occur (Bonin et al. 2004).

An additional potential problem with both microsatellites and AFLP is the risk of size homoplasy: mutations that are not creating length differences (substitutions) create alleles that are not identical by descent, but which are inseparable in fragment length analyses (Estoup and Cornuet 1999, Bensch and Åkesson 2005). Furthermore, for microsatellites, all mutation models except the IAM model predict back-mutations which produce alleles already found in the population, but which are not identical by descent (Estoup et al. 2002). The high number of alleles for microsatellites, and loci for AFLPs, hopefully dilute the effects of homoplasy, and also scoring errors.

Statistical methods

One way of partitioning the statistical tests used in this thesis is between “a priori” and “a posteriori” tests or “looking for structure without prior knowledge or hypotheses of patterns” and “testing a hypothetical structure”, for example based on geographic sampling-localities or predefined subspecies. I have used both approaches, although I find the “a priori” analyses most interesting – then there are no prejudices that can lead to false conclusions. Besides, hypotheses of genetic structure are often based on morphological or

geographical patterns, and in cases when underlying genetic structure does not correspond to these patterns, you may miss interesting patterns when testing these hypotheses.

Another division among statistical tests is between Maximum likelihood and Bayesian statistics. Traditional statistical methods are based on maximum likelihood: you assume a model, and calculate the probability of your data, given that model. A more recently developed approach is Bayesian statistics. Here you calculate the probability of a model, given the data. Said in another way, Bayesian statistics is "a method of statistical analysis that begins with prior distributions for the model parameters and updates these based on observed data to arrive at a posterior probability distribution" (Manel et al. 2005). Both methods can use simulations to estimate parameters, either those that best fit your data (Bayesian), or those that maximise the likelihood, given the data. For this, Markow chains are frequently used to search parameter landscapes to avoid the cumbersome procedure of exact calculations when the number of possible partitions is too large.

Coalescence theory is a mathematical framework in which one can do tests about gene genealogies. The theory concerns the phylogenetic relationships between DNA-sequences found in present populations by tracing the genealogical branching process backwards in time. Parameters concerning time since common ancestor (coalescence time) and effective population sizes are estimated (Avise 2000). In addition, coalescence approaches can reveal historical population size changes. If a population experienced a sudden population expansion at a point in time, many of today's genealogical lineages will coalesce at that point. If frequencies of pairwise differences between genotypes in the population are plotted, the x-axis can be seen as both number of base pair-differences *and* as relative time since coalescence of two sequences. A large degree of difference between two sequences corresponds to a long time since coalescence. The population expansion will then be observed as a wave in the frequency distribution. These distributions are called mismatch distributions. It is possible to calculate the expected distributions under constant and growing population sizes, and test if there are differences between the observed and expected distributions.

Another way to test for population size differences is to compare different estimates for expected polymorphism (θ). Polymorphism at a locus depends on effective population size (N_e) and mutation rate (μ) and can be expressed as $4N_e\mu$ for autosomal loci of diploid organisms. Polymorphism also depends on whether the mutants are selectively neutral or not (Li 1997). Under selection and changes in population size, different estimates of θ are affected differently. For example, Π (average number of nucleotide differences between

two sequences randomly chosen from the population) takes into account the variance of sequences, whereas K (number of segregating sites) ignores this. Tajima's D test of neutrality (Tajima 1989) takes advantage of this difference. Ramos-Onsins and Rozas' (2002) R_2 value for detecting population growth is based on the difference between the number of singleton mutations and the average number of nucleotide differences. Other tests (*e.g.* Fu and Li's G- and F-tests) estimate θ based on coalescence theory. I have performed all these tests in my studies, but in most cases I have found it confusing to give all test statistics, and have chosen to give only the R_2 value as this was shown to be most powerful test for detecting population growth for small sample sizes (Ramos-Onsins and Rozas 2002).

It is necessary to be cautious when calculating effective population sizes and population size changes according to these methods and theories because these factors are connected, to each other, and to other factors like gene flow (P Palsbøll pers. comm.). It is also important to keep in mind that the described calculations often only give relative estimates because mutation rates and generation times often are not known.

Results

Paper I: Phylogeography in snowy owls

Snowy owl individuals from three well geographically separated regions on the northern Hemisphere (Scandinavia, eastern Russia and North America) did not differ genetically according to three variable genetic markers; two mitochondrial and one nuclear. For the less variable loci; Cyt *b* (mtDNA; six haplotypes among 40 individuals) and BRM15 (Z-chromosome intron; two haplotypes), the haplotypes differed by maximum three mutations, and there were small differences in frequencies between different areas. For the unexpectedly variable control region (33 haplotypes), almost no haplotype was shared between individuals, and related haplotypes were not consistently found in the same place. Conclusively, the snowy owls seem to constitute one panmictic population today. Mismatch distribution analyses and investigation of the control region haplotype network indicated several historical cycles of isolation and fusion events for separate populations.

The level of genetic diversity in snowy owl at the analyzed loci was found to be high compared to owl species breeding in the temperate region; higher than in eagle owl

(*Bubo bubo*) and tawny owl (*Strix aluco*), and at the same level as in Tengmalm's owl (*Aegolius funereus*). The former two are relatively sedentary; Tengmalm's owls are nomadic to some extent, although not as much as the snowy owl.

Paper II, III and IV: Phylogeography, taxonomy and conservation of dunlins

Dunlins in Western Palearctic and East Greenland were in Paper II shown through analyses of microsatellites and AFLPs to not constitute genetically separable groups, despite presumably constituting four subspecies. Rather, a general pattern of isolation by distance was discovered for the microsatellites. AFLP did not give any phylogeographic signal. In paper III, the previously described pattern of two mtDNA control region clades was confirmed. Also the difference between Icelandic dunlins and all other populations in frequency and composition of haplotypes was confirmed. The Z-chromosome intron VLDLR-9 did not give any phylogeographic signal. Threatened dunlin populations found mainly in the Baltic Sea area were, based on four genetic markers, shown to not be genetically deprived or inbred, as compared to the other investigated populations. Similarly, the Svalbard population was not inbred or genetically deprived according to analyses in paper IV. Furthermore, using mtDNA control region sequences and microsatellites, I found that the Svalbard population was genetically similar to East Greenland, Iceland and the Baltic populations. Taxonomically, this result connects Svalbard dunlins to the *schinzii* and *arctica* subspecies, rather than to *alpina* or *centralis*.

Bill lengths were measured in South Norwegian populations and in Svalbard and related to measures taken from the literature from other populations, in Paper III and IV. Males of *alpina* and *schinzii* differed significantly in bill lengths, but there was no abrupt pattern corresponding to the subspecies taxonomy (Fig. 7).

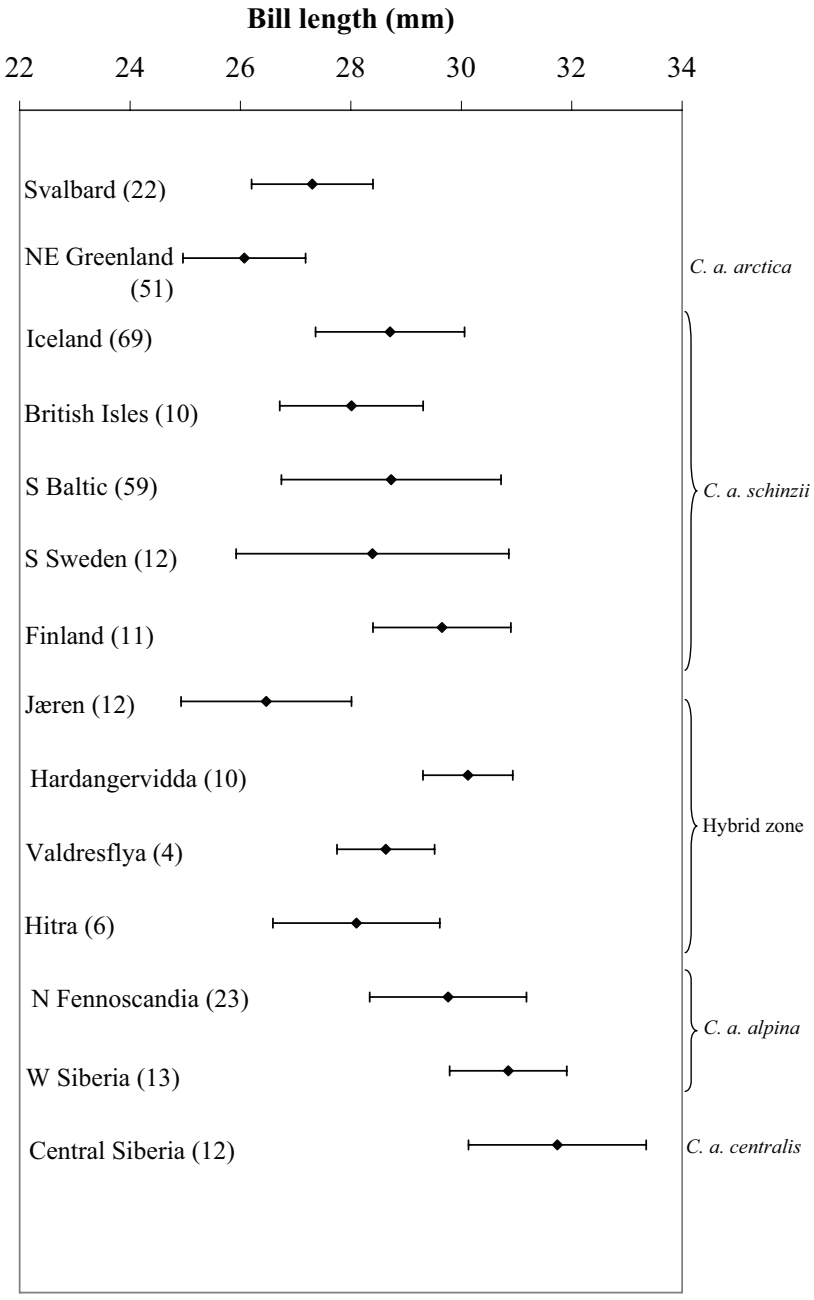

Figure 7 Bill lengths in dunlin (*Calidris alpina*) males in Western Palearctic and East Greenland. All data except for measures of the South Norwegian populations and Svalbard are taken from Greenwood (1986). Bars represent one standard deviation.

Paper V: The redpoll complex – still a taxonomic enigma

Neither mtDNA control region sequences nor microsatellites revealed any structuring among redpoll individuals from the three putative species *C. flammea*, *C. hornemanni* and *C. cabaret* when testing for structure without prior hypotheses. When testing for differences between the species, however, small, yet significant F_{ST} values were found. *C. f. rostrata* did not differ from *C. f. flammea*. The outgroup – the twite – was well separated from the redpolls, and the two twite subspecies were also well separated, both in mtDNA and microsatellites. Only one individual of *flavirostris* grouped with *rufostrigata* in one microsatellite analysis. The twite subspecies were in other words much more differentiated than were the redpoll species.

A mismatch distribution revealed a signature of a historic exponential population growth, as has previously been demonstrated by Ottvall et al. (2002).

Discussion

The main finding in this thesis is a low level of genetic structure in three studied bird species and species complexes revealed by neutral genetic markers. For snowy owls, no predefined structure existed, *e.g.* in form of subspecies, and the finding of birds from three regions being genetically very similar was not particularly surprising. For the two other taxa, however, the low levels of structure were unexpected. For dunlins, the genetic patterns found did not correspond to morphologically and in part geographically separated groups or designated subspecies. Rather, the prevalent phylogeographic structure was a pattern of isolation by distance. The redpoll species were found to differ slightly in allele frequencies, but to a much lesser extent than is expected for species. The investigated subspecies were not differentiated.

Adaptive versus neutral variation

The taxonomic delimitation of dunlin subspecies and redpoll species is phenotypically based, as is most often the case in avian taxonomy. Dunlin subspecies in Western Palearctic and East Greenland have been shown to differ in biometric measures (*i.e.* bill and wing length), plumage, breeding habitat, wintering places and time of moult (Soikkeli

1966, Cramp and Simmons 1983, Greenwood 1986, Hayman et al. 1986, del Hoyo et al. 1996, Engelmoer and Roselaar 1998). The redpoll species have been shown to differ in plumage, biometric measures, physiology, timing of migration, nest habitats, diets, vocalizations and behaviour (Brooks 1968, Molau 1985, Knox 1988, Herremans 1989, Seutin et al. 1992, Lifjeld and Bjerke 1996, Knox et al. 2001). These phenotypic differences presumably represent adaptive traits, and adaptive traits are important in designation of conservation units because they indicate capacity for response to environmental change, and whether fitness may be maintained (*e.g.* Frankham et al. 2004). Phenotypic characters are however not always adaptive traits. First, not all morphological variation is genetically based. Morphological polytypism within single species can arise from ecophenotypic mechanisms, in which for example different food gives different plumage (Brush 1981). Diet has been shown to affect carotenoid-based pigmentation in great tits (Slagsvold and Lifjeld 1985) so that birds feeding in different habitats had different degrees of yellowish colouration. Diet has also been shown to affect red colouration in redpolls. Molau (1985) kept birds in cages during the moulting period and concluded that the intensity of red was highly correlated with food based on the fact that his birds lost much more of the redness in their plumage in the winter feathers than what normally happens in nature. However, redness is scarcely used as a diagnostic character in redpolls (Knox 1988) and thus does not pose a problem in this case. Second, morphological polytypism could be caused by one major locus with pleiotropic effects (one gene affects several phenotypic characters) or epistatic effects (two or more gene loci interact and affect the phenotype in a stronger way than two non-interacting loci would have) (Seutin et al. 1995). Alternatively, one locus (without pleiotropic effects) may produce polymorphism through conspicuous differences, as for example in bananaquit (*Coereba flaveola*), snow goose (*Anser c. caerulescens*) and Arctic skua (*Stercorarius parasiticus*), where melanistic polymorphisms have been shown to be controlled by one single locus (Mundy 2005). Pleiotropic or epistatic effects causing morphological differences are possible in both dunlins and redpolls, but it seems unlikely that all the mentioned corresponding characters are affected by such mechanisms. Generally, the differences reported between dunlin and redpoll subspecies and species therefore most likely represent selectively active traits.

Although adaptive traits are considered important, they do not always reveal the genetic history and evolutionary potential of taxonomic groups. Information about these

aspects is important, not only for conservational decisions, but also for studies of evolutionary processes, like speciation.

For the snowy owl I did not only show that the birds seem to exchange genes across their entire range. I also showed that genetic variation is considerable at a neutral locus (mtDNA control region), and I speculate that the Pleistocene glacial cycles have facilitated divergence leading to this high variation level. This has implications for conservation of threatened snowy owls in Western Palearctic because it means that they are not genetically inbred or deprived.

The phylogeographic pattern revealed for the dunlins was a clinal rather than abrupt pattern of variation; a gradual change of allele frequencies was found across the investigated region for two markers. Kraaijeveld and Nieboer (2000) suggested that the biometric differences between dunlin subspecies in Europe are of recent origin, and that different ecology has exposed the subspecies to different selection pressures. Rapid evolution on morphological characters, creating differences not yet reflected in neutral DNA, has been suggested to be responsible for the patterns found in for example yellow wagtail (*Motacilla flava*, Ödeen and Björklund 2003), bluethroat (*Luscinia svecica*, Questiau et al. 1998, Zink et al. 2003, Johnsen et al. 2006), common grackle (*Quiscalus quiscula*, Zink et al. 1991), song sparrow (*Melospiza melodia*, Zink and Dittmann 1993) and swamp sparrow (*Melospiza georgiana*, Greenberg et al. 1998). In these taxa, two or more subspecies or species are described based on sexually or naturally selected plumage patterns, while genetic studies have revealed a lower number of groups. Also for redpoll species, among which very little genetic structure appeared, Ottvall et al. (2002) suggested a recent divergence of morphological diagnostic characters in their study on *C. flammea* and *C. cabaret*.

However, ongoing gene flow may also explain the low levels of genetic structure in dunlins and redpolls – strong selection may produce differences in morphologic traits despite gene flow between groups of individuals. A strong selection pressure counteracting gene flow has been shown for example in little greenbulls (*Andropadus virens*) breeding in different ecotone habitats in the rainforest (Smith et al. 1997), and in crossbills (*Loxia* spp.). Crossbills comprise several species and subspecies worldwide which are morphologically differentiated, but genetically similar within Palearctic and North-America respectively (Questiau et al. 1999, Piertney et al. 2001). Observations of mixed pairs that fledged young proved gene flow between taxa in Scotland (Summers et al. 2007). The differing habitat preferences of the redpoll species may have provided differing

selection pressures strong enough to counteract potential gene flow taking place among the groups. The lack of observations of mixed species pairs or hybrid offspring (Molau 1985, Knox 1988) does not mean they do not occur. In dunlins, gene flow of mtDNA molecules has obviously occurred after the last ice ages, as seen from the mixture of EUR and SIB haplotypes in northern Europe. Which mechanism – gene flow or recent divergence of phenotypic characters – should be invoked to explain the lack of clear genetic groups in Western Palearctic dunlins and redpolls is difficult to say without further studies. However, which mechanism is the dominant one determines evolutionary potential and future development for the taxa.

Different histories in the studied taxa

The three investigated species and species complexes in this thesis differ in behaviour and ecology, and this may explain the apparently different histories. The snowy owl breeds in the most extreme arctic habitat, whereas the dunlin and the redpoll breed also in temperate regions. This means that their habitats during the Pleistocene ice ages must have been differently affected. As seen from the mtDNA haplotype network, and possibly also from the skewed mismatch distribution for the snowy owl data (Paper I), the snowy owls seem to have been isolated in refugia (where differences developed) one or several times, but have since the ice ages fused and mixed completely due to an increase in available habitat and their nomadic behaviour. The dunlins seem to have been separated in different ice age refugia (Wenink et al. 1996), but they have not mixed to as large an extent as have the snowy owls later on. This may probably be explained by their much higher degree of site fidelity. Snowy owls are highly nomadic due to the cyclic abundance of their prey (Alerstam 1990, Cramp and Simmons 1994), whereas dunlins are faithful to their breeding sites (Soikkeli 1970, Jackson 1994, Thorup 1999). Redpolls are nomadic like the snowy owls – the snowy owls follow rodent peaks, the redpolls follow seed peaks. This facilitates high levels of gene flow in the redpolls. And indeed, redpolls, like snowy owls, did not show any genetic structure. It has been suggested that the different redpoll taxa were isolated in different Pleistocene refugia (Johansen 1958). However, the time spent in isolation must have been shorter than for for example the dunlins, as there are no well separated mtDNA haplotype clades in the redpoll dataset corresponding to the dunlin clades (Wenink et al. 1996). Furthermore, there is overlap between redpoll species in the closely related haplotype clades that do exist, only allele or haplotype frequencies differ.

Neutral genetic markers and conservation

Neutral genetic markers do not always give the phylogeographic resolution we need. For the dunlins, AFLPs and VLDLR-9 did not reveal the desired phylogeographic resolution level despite a decent level of diversity. This may be due to high levels of homoplasy, or perhaps more likely, too few AFLP markers (Paper II) and high levels of male gene flow or constraints on variation in introns because of selection pressures on the VLDLR-gene (Paper III). However, all neutral genetic markers showing a certain degree of polymorphism may be used for inferences of genetic diversity within populations. I found no evidence for inbreeding or genetic deprivation in threatened or not-threatened populations in any study. However, demographic factors indicate that at least the dunlin populations may become inbred or genetically deprived in the future, as human disturbances will probably lead to further habitat destruction and isolation of the small populations. This may also happen for snowy owls and redpolls, as the impacts human activities have on their habitats increase.

A farewell to subspecies?

Humans are used to think in categorical terms, and the clinal variation found with genetic markers in the investigated taxa may pose problems for for example management decisions. However, the reason for this kind of categorical thinking is not necessarily based on abrupt patterns of morphological variation, but may rather be a feature of our minds – it has been adaptive for us to categorize. This urge to classify is deeply rooted within us; already in ancient times, the philosophers were keen to find a natural order (cosmos). Furthermore, several religions' descriptions of Genesis include formation and naming of species (*e.g.* Genesis, 2 19-20). As a consequence of this urge to categorize, we tend to categorize also where there are no good categories, and I think this over-classification is a rather common phenomenon.

An excellent example of clinal variation in nature is found in so-called ring species. In some cases, gradual differentiation can proceed within a species around an obstacle to gene flow, like the Arctic Ocean or a non-inhabitable mountain plateau, and at the meeting point of the two ends of the distribution, the individuals may be so different that they cannot reproduce anymore. The populations at the ends should then be considered different species, but because they are connected through interbreeding populations, they are

considered to belong to the same species, called a ring species (Mayr 1942, Cain 1954). Such patterns have been demonstrated for example in the greenish warbler (*Phylloscopus trochiloides*, Irwin et al. 2001), breeding around the Tibetan plateau, and the Californian newt (*Ensatina eschscholtzii*), breeding around the Central Valley in California (Moritz et al. 1992). The same kind of clinal variation can be found in other species with continuous distribution, but in which the distribution ends do not meet, for example across Palearctic or Nearctic. A difference compared to ring species is that the clinal variation in these cases does not pose a problem in species taxonomy, and one can easily call the group one species. Sometimes though, such species are further delimitated into subspecies despite the continuous variation, and these cases are good examples of categorization taken somewhat too far. The dunlin subspecies, for example, are considered to have a well founded morphological basis. However, several characters used to discern among dunlin subspecies are of a clinal character, for example body size and plumage (von Blotzheim et al. 1975, Cramp and Simmons 1983), and bill length (Greenwood 1986, Engelmoer and Roselaar 1998, Paper III, Fig. 7). Some studies on morphological variation in dunlins have found clusters of populations that correspond to the subspecies in Western Palearctic (Greenwood 1986, Engelmoer and Roselaar 1998), but it is possible that for example differences found between *C. a. schinzii* and *C. a. alpina* are consequences of the lack of samples in central Scandinavia, where dunlins do breed, in these studies. Biometric measures intermediate between *C. a. alpina* and *C. a. schinzii* have been found in southern Norway (Haftorn 1971, Kålås and Byrkjedal 1981) and northern Sweden (Swanberg 1939). The taxonomic situation of dunlins in Western Palearctic illustrates two points regarding clinal variation. First, patchy sampling may lead to erroneous conclusions on clusters of individuals (Crandall et al. 2000). Second, when testing for differences between predefined clusters within a taxon that actually form continuous distributions with clinal variation, you *will* find differences, but this should not be taken as evidence for the presence of clear clusters of individuals.

Does this view of clinal variation rather than categories being the norm in nature, at least within species, mean a farewell to for example subspecies? I do not think so. I think it is of great importance to acknowledge the variation that does exist, and maybe the delimitation of subspecies or Evolutionarily Significant Units (Moritz 1994) is the best way to handle that variation, given their practical value (Moritz 2002). However, I do consider clinal variation to be a general issue in taxonomy that needs to be addressed in conservation and evolutionary studies in particular. I thus suggest a farewell to the idea

that most variation is discrete. We need to embrace the reality of continuous variation, and incorporate it into conservational politics and evolutionary studies.

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