UNIVERSITY OF ICELAND

Faculty of Science

Department of Biology

Do herring gulls (Larus argentatus) and glaucous gulls (Larus hyperboreus) hybridize in Iceland? A study on phenotypic and genetic variation

Freydís Vigfúsdóttir

Research paper for the degree of MSc (45 units)

June 2007

Do herring gulls (Larus argentatus) and glaucous gulls (Larus hyperboreus) hybridize in Iceland? A study on phenotypic and genetic variation

Freydís Vigfúsdóttir

Research paper for the degree of MSc (45 units)

Supervisors and MSc committee:

Snæbjörn Pálsson

Agnar Ingólfsson

University of Iceland

Faculty of Science

Department of Biology

June 2007

Kynblandast silfurmáfar (Larus argentatus) og hvítmáfar (Larus hyperboreus) á Íslandi? Rannsókn á svipfars- og erfðabreytileika

Freydís Vigfúsdóttir

45 eininga ritgerð til MSc prófs

Leiðbeinendur & MSc nefnd:

Snæbjörn Pálsson

Agnar Ingólfsson

Háskóli Íslands

Raunvísindadeild

Líffræðiskor

Júní 2007

I declare that this thesis is supported by my research work, written by myself and has not as a part or as a whole been published before due to higher educational degree.

Hér með lýsi ég því yfir að ritgerð þlessi er byggð á mínum eigin athugunum, er samin af mér og að hún hefur hvorki að hluta né í heild verið lögð fram áður til hærri prófgráðu.

Freydís Vigfúsdóttir

Contents

Abstract

Avian hybrids are widespread and many bird species are known to have hybridized in nature. Hybridization among gull species has been reported from many areas. Presumed recent hybridization reported for glaucous gull (*Larus hyperboreus*) and herring gull (*Larus argentatus*) in Iceland, after the colonization of the latter around 1925, has been debated. Advances in molecular genetics have enabled a greater precision in examination of hybridization.
The aim of this thesis is to evaluate whether and to what extent hybridization occurs among glaucous gulls and herring gulls in Iceland as reflected by morphological traits and genetical markers in samples obtained over a forty years in Iceland.
The thesis is based on two papers.

In the first paper, analysis of microsatellites and sequence variation of the mtDNA cytochrome *b* region were applied to detect whether signs of hybridization exist between the two species in Iceland and to infer genetic structures of populations within the two species. Museum specimens of both species were analysed together with samples from contemporary populations to assess genetic variation in time and space. Furthermore, specimens from Greenland were analyzed, and compared with published sequences for a more detailed phylogeographic view. A distinctive difference was detected between the Icelandic and Greenland *hyperboreus*. Extensive mtDNA haplotype sharing was detected between the two species in Iceland with unique existence of *hyperboreus* haplotypes in *argentatus* and, to a lesser degree, vice versa. Since the first time period of the study (1964 – 1973), mtDNA nucleotide diversity increased in *argentatus*. Microsatellite allele variation was shared between the species in Iceland and differentiation by private alleles was very limited. Genetic analysis showed clear distinction between most *hyperboreus* and *argentatus*-like birds, and among samples within both groups. The analysis also revealed a peculiar *hyperboreus* group clustering among *argentatus.* Admixture analysis indicated a number of individuals that were more likely to be assigned to the other species. Results from both nuclear and mitochondrial markers confirm previously debated hybridization in Iceland.

In the second paper morphological variation of the two species were compared with genetical information based on microsatellite and mtDNA data, in order to analyse the nature of hybridization and extent of introgression among the species. Comparisons of both single traits and multivariate analysis pointed to hybridization and introgression. Putative hybrid individuals were in some cases intermediate in their morphology, in other cases they presented one of the parental type, and could only be identified with genetic markers. The extensive hybridization observerd during the first period in southeast Iceland had not had lasting effects of the populations studied. The study supported the fact that phenotypic intermediacy can be indicative of hybridization, as has been demonstrated in numerous studies on various gull species, and proved to be more effective when morphometric traits are analyzed together. The results also showed that genetic analysis added more details and precision in describing the hybrid scenario.

Ágrip

Kynblöndun hefur verið lýst meðal margra fuglategunda og vísbendingar um kynblöndun ýmissa máfategunda hafa komið víða að, en þær hafa verið rannsakaðar í mismiklum mæli. Silfurmáfar (*Larus argentatus*) námu land á Íslandi í kringum 1925. Með samanburði á útlitsbreytileika íslenskra silfurmáfa við silfurmáfa annarsstaðar í Evrópu setti Agnar Ingólfsson þá kenningu fram, 1970, að silfurmáfar hér á landi hefðu kynblandast við hvítmáfa (*Larus hyperboreus*). Hvítmáfar hafa útbreiðslu umhverfis norður heimskautið en silfurmáfar hafa suðlægari útbreiðslu. Meginrökin fyrir kynblönduninni voru þau að stór hluti silfurmáfaa á Íslandi sýndu ýmis einkenni hvítmáfa,. Þessi kenning um kynblöndun var dregin í efa af kanadískum fuglafræðingi R. R. Snell sem taldi að breytileikinn meðal silfurmáfa á Íslandi endurspeglaði breytileika innan silfurmáfa og landnáms fárra einstaklinga. Framfarir á sviði sameindaerfðafræði hafa skapað betri og nákvæmari leiðir til rannsókna á kynblöndun og gefið möguleika á að fá lausn á þessari deilu. Markmið verkefnisins var að meta hvort og hvers eðlis kynblöndun er milli hvítmáfa og silfurmáfa á Íslandi, í ljósi breytileika í útlitseiginleikum og erfðamörkum. Rannsökuð voru sýni sem safnað var á yfir fjörutíu ára tímabili. Eldri sýni beggja tegunda eru varðveitt á Náttúrufræðistofnun Íslands. Verkefnið er sett fram í tveimur handritum.

Í fyrra handritinu er því lýst hvernig greining örtungla og raðgreininga á cytochrome *b* geni í erfðaefni hvatbera er beitt til að meta hvort kynblöndun greinist milli silfurmáfa og hvítmáfa á Íslandi. Samsetning stofna innan tegunda var einnig athuguð, bæði í tíma og rúmi. Að auki voru sýni tekin á Grænlandi og niðurstöður rannsóknarinnar borin saman við hliðstæðar DNA raðir úr rannsóknum annarra til að fá betri heildarmynd af landfræðilegum breytileika innan tegundanna. Greinilegur munur fannst milli íslenskra og grænlenskra hvítmáfa. Á Íslandi deildu tegundirnar mörgum af þeim arfgerðum sem fundust og erfðaefni sem var sértækt fyrir hvítmáfa á Íslandi fannst í íslenskum silfurmáfum, og öfugt. Breytileiki hvatbera hefur aukist í silfurmáfum frá fyrstu sýnatökum á sjöunda áratug síðustu aldar. Athugunin sýndi skýra aðgreiningu milli flestra hvítmáfa og silfurmáfa, auk þess mun milli hópa innan tegunda. Þó greindist sérstakur hvítmáfshópur sem hópaðist meðal silfurmáfa. Nánari greining á arfgerðum einstakra máfa gaf til kynna að margir þeirra, sérstaklega silfurmáfar, voru líklegri til að flokkast til hinnar tegundarinnar. Niðurstöðurnar staðfesta áður umdeilda kynblöndun meðal hvítmáfs og silfurmáfs á Íslandi.

Í seinna handritinu er greint nánar frá rannsókn á sýnum frá Íslandi. Útlitsbreytileiki beggja tegunda var borinn saman við erfðabreytileika sem byggði á örtunglum og hvatbera erfðaupplýsingum, til þess meta eðli kynblöndunarinnar milli tegundanna. Samanburðir byggðir á einstökum útlitsmælingum og fjölbreytugreiningu benda til kynblöndunar og genaflæðis milli tegundanna. Meintir kynblendingar einkenndust í sumum tilfellum af millistigi útlitsbreytileika hvorrar tegundar, en í öðrum tilvikum greindust þeir aðeins með erfðamörkum. Hin víðtæka kynblöndun sem greindist á fyrsta tímabili rannsóknarinnar (1964-1973) á suðausturlandi hefur ekki greinst í eins miklum mæli í seinni tíma sýnum sem rannsökuð voru. Rannsóknin styður að millistig í útlitsbreytum geta verið vísbendingar um kynblöndun en margar rannsóknir hafa nýtt sér millistig sem þessi til greiningar á kynblöndun ýmissa máfategunda. Greining blöndunar var nákvæmari eftir því sem fleiri breytur voru notaðar í fjölbreytugreiningu. Rannsóknin sýndi einnig að með erfðaupplýsingum má lýsa tilfelli kynblöndunar af meiri nákvæmni og áreiðanleika.

Chapter I

The presumed hybridization of glaucous gull (Larus hyperboreus) and herring gull (Larus argentatus) in Iceland: mitochondrial and microsatellite data

The presumed hybridization of glaucous gull (*Larus hyperboreus*) and herring gull (*Larus argentatus*) in Iceland: mitochondrial and microsatellite data

FREYDÍS VIGFÚSDÓTTIR, SNÆBJÖRN PÁLSSON and AGNAR INGÓLFSSON
Department of Biology, University of Iceland, Sturlugata 7, 101 Reykjavík, Iceland

Abstract

The herring gull, *Larus argentatus,* colonized Iceland around 1925 where the glaucous gull, *Larus hyperboreus* was already present. Microsatellite and sequence analysis of the mtDNA cytochrome *b* region were applied to infer genetic structure of the population and to detect whether signs of hybridization exist between the two species in Iceland. Museum specimens of both species were analyzed together with samples from contemporary populations to assess genetic variation in time and space. Furthermore, specimens from Greenland were analyzed for a more detailed phylogeographic view. A distinctive difference was detected between the Icelandic and Greenland *hyperboreus*. Extensive mtDNA haplotype sharing was detected between the two species in Iceland with unique existence of *hyperboreus* haplotypes in *argentatus* and vice versa. Since about 1985, mtDNA nucleotide diversity increased in *argentatus* while it decreased in *hyperboreus*. Microsatellite allele variation was shared between the species in Iceland and differentiation by private alleles was very limited. Genetic analysis showed clear distinction between most *hyperboreus* and *argentatus*-like birds, and among samples within both groups. The analysis also revealed a peculiar *hyperboreus* group clustering among *argentatus.* Admixture analysis indicated a number of individuals that were more likely to be assigned to the other species. Results from both nuclear and mitochondrial markers confirm previously debated hybridization in Iceland.

Keywords: Gulls, hybridization, microsatellites, mtDNA, cytochrome *b*, phylogeography.

Introduction

Species distributions and genetic population structure have been found to reflect climatic oscillations during the Quaternary glacial eras (Hewitt 2000; Newton 2003). Ancestral populations could split up during glacial periods and evolve in allopatry in distinct refugia (Hewitt 1996). Following the retreat of the glaciers, populations of several species have expanded and formed secondary contact zones (Barton and Hewitt 1985; Hewitt 2004), often involving various forms of hybridization (Grant and Grant 1992).

Avian hybrids are widespread and more than 9% of all bird species are known to have hybridized in nature, although this is more common in some taxa than others (Grant and Grant 1992; Randler 2002). Hybridization among gull species has been reported from many areas (McCarthy 2006), e.g. *Larus (argentatus) smithsonianus* x *Larus glaucoides* in the Mackenzie Delta, Canada (Spear 1987), *Larus glaucescens* x *Larus occidentalis* on the Pacific coast of North America (Bell 1996), *Larus argentatus* x *Larus fuscus* in western France (Yesou 1991), *Larus argentatus* x *Larus cachinnans* in European Russia (Panov and Monzikov 1999), and *Larus argentatus* x *Larus glaucoides* in Iceland (Ingólfsson 1970; Randler 2002). It has been suggested that this widespread hybridization could be explained by incomplete reproductive isolation among several white-headed gulls, which has evolved more rapidly between some lineages than others, possibly reflecting two main glacial refugia (Crochet *et al.* 2003; Crochet *et al.* 2002; Liebers *et al.* 2004).

The glaucous gull (*Larus hyperboreus*) and the European herring gull (*Larus argentatus*) came in to contact in Iceland when the latter colonized Iceland around 1925. Ingólfsson (1970, 1987) described hybridization among the two species based on morphological variation. Apparently pure glaucous gulls predominated in western Iceland while apparently pure herring gulls and hybrids were common in southern and eastern Iceland. Studying allozyme and morphological variation, (Snell 1991a, b) argued against hybridization, claiming that the variation found in herring gulls in Iceland simply reflected natural variation within the species (see also rebuttal by Ingólfsson 1993).

Advances in molecular genetics have enabled a greater precision in examination of hybridization, and have helped to recognize hybridization as a significant process in population processes (Rhymer and Simberloff 1996; Vallender *et al.* 2007). Previous studies of the genetics of the herring gull have failed to detect whether hybridization was taking place

(Snell 1991a). Studies by Bell (1996) and (Crochet 2000) indicated that the situation was more complex than simple surveys of plumage phenotypes or allozyme variation had shown. More recent genetic studies on herring gulls and related species have confirmed that the phylogeny of large white–headed gulls is much more complex than previously thought and includes instances of hybridization (Crochet *et al.* 2003; Crochet *et al.* 2002; Liebers *et al.* 2004; Liebers-Helbig *et al.* 2006).

Here we evaluate whether hybridization occurs among glaucous gulls (*hyperboreus*) and herring gulls (*argentatus*) in Iceland. The analysis is based on variation at five microsatellite loci and sequence variation in mtDNA (cytochrome *b*). We also assessed possible genetic changes in time and space.

Material and methods

Samples

Samples were obtained from various localities in Iceland and additionally from Kulusuk, (K) in Greenland (Figure 1). According to Ingólfsson (1970), the location in west Iceland (V) was dominated by *hyperboreus*-like birds, while the location in east Iceland (A) was dominated by *argentatus*-like birds and apparent hybrids. In the location in south east Iceland (SA), both types as well as hybrids were common.

Figure 1. Main sampling locations of gulls investigated. Letters denote location, listed in Table 1.

Samples originated from three time periods, 1 - 3 and are listed in Table 1: species, period, and location. Sampling was carried out in the field 2005 – 2006 (period 3) by shooting or Cannon netting, by first attracting gulls by putting out bait near breeding colonies. Other samples were obtained from specimens at The Museum of Natural History in Reykjavik, collected by Ingólfsson in 1964 – 1973 (period 1) and Snell in 1985 – 1986 (period 2) in breeding colonies. For the purpose of the analysis, gulls were assigned to two groups on the basis of primary pattern, those scoring 0.0 - 1.0 in “hybrid index” (see Ingólfsson 1970) being

called *hyperboreus*, and those scoring between 1.1-5.0 being termed *argentatus.* This grouping is artificial and many of these birds may possibly be of hybrid origing (Vigfúsdóttir *et. al.* in prep.). For comparison, Iceland gulls *Larus glaucoides* were also collected in time period 3. Iceland gulls breed in Greenland and overwinter in Iceland.

Table 1. Number of individuals sampled from each site in three time periods*. Letters in sample name are as follows: Species, time period, sampling location. Letters of location correspond to figure 1.

Samples	Species	Sampling sites	Latitude	Longitude	n
A1A	*Argentatus*	Hromundarey	64°35´35´´	14°18´58´´	46
–ıı–	–ıı–	Horn	64°14´50´´	14°59´53´´	13
–ıı–	–ıı–	Skrudur	64°54´13´´	13°38´17´´	2
–ıı–	–ıı–	Reykjanes	64°00´55´´	22°40´21´´	3
–ıı–	–ıı–	Hjorleifshofdi	63°25´28´´	18°45´38´´	1
A2A	–ıı–	Skrudur	64°54´13´´	13°38´17´´	37
A3A	–ıı–	Reydarfjordur	64°56´03´´	13°41´36´´	25
A3V	–ıı–	Grundafjordur	64°55´31´´	23°15´40´´	3
–ıı–	–ıı–	Reykjanes	64°00´55´´	22°40´21´´	4
H1V	*Hyperboreus*	Bjarnarhafnarfjall	64°59´07´´	23°01´07´´	45
–ıı–	–ıı–	Bulandshofdi	64°56´39´´	23°28´48´´	5
–ıı–	–ıı–	Reykholahreppur	65°30´21´´	22°17´16´´	3
H2V	–ıı–	Bjarnarhafnarfjall	64°59´07´´	23°01´07´´	26
–ıı–	–ıı–	Skrudur	64°54´13´´	13°38´17´´	1
H3V	–ıı–	Grundafjordur	64°55´31´´	23°15´40´´	65
–ıı–	–ıı–	Reydarfjordur	64°56´03´´	13°41´36´´	1
H1A	–ıı–	Horn	64°14´50´´	14°59´53´´	2
–ıı–	–ıı–	Hromundarey	64°35´35´´	14°18´58´´	6
H3K	–ıı–	Kulusuk	65°34´25´´	37°10´18´´	19
G	*Glaucoides*	Kulusuk	65°34´25´´	37°10´18´´	4
–ıı–	–ıı–	Reydarfjordur	64°56´03´´	13°41´36´´	3
					314

*Time period 1: 1964-1973 sampled by Ingólfsson,
period 2: 1985-1986 sampled by Snell,
period 3: 2005-2006 sampled by authors.

Molecular analysis

DNA was extracted from feathers in 300 µl 6% chelex solution containing 3 µl proteinase K (0.5 mg/mL). After incubation (2 h, 65°C, with agitation at 1000 rpm) samples were heated to 95°C for 5 min, to inactivate the proteinase, and centrifuged for 5 min at 3000 rpm. After cooling (30 min, 4°C), the resulting sample was used directly for PCR amplification.

A 971 bp fragment of the mtDNA cytochrome *b* gene was amplified using primers L14967 and H15938, as in Crochet *et al.* (2003). L and H refer to the light and heavy strands respectively, and the numbers refer to the position of the 3' base in the domestic fowl (*Gallus gallus*) mtDNA sequence (Desjardins and Morais 1990). For the museum specimens, two smaller overlapping segments of the cytochrome *b* gene were amplified separately, using

primers L15440 (5'GCCAAACCCTCGTAGAATGA-3'), with H15938, and H15619 (5'GTAGGGGTGGAATGGGATTT-3') with L15008 (Crochet *el. al.* 2003), covering the same region of the cytochrome *b* gene. Polymerase chain reaction (PCR) amplifications were carried out in 10 μl volume containing 1X amplification buffer/ 0.09U of Taq DNA polymerase, 1.5 mM $MgCl_2$, 1 mM of each dNTP, and 1 pM of each primer. Cycling conditions were 94°C for 40 s, 56°C for 30 s and 72°C for 60 s for 30 cycles for primers L14967-H15938. For primers used on museum specimens cycling conditions were 94°C for 60 s, 53°C for 30 s and 72°C for 60 s for 38 cycles. Negative controls were always included with each PCR reaction and presence or absence of amplification in all samples was visualized under UV light in 1.5% agarose with ethidium bromide. All plastic and metal materials were UV-irradiated prior to use.

Prior to sequencing, excess primers and nucleotides were enzymatically removed from PCR amplification products using a mixture of exonuclease I and antarctic phosphatase (New England BioLabs). Cycle sequencing was carried out using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), using a step cycle profile: 5 min at 94°C followed by 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 58°C) and extension (30 s at 72°C). The product of the sequencing reaction was precipated with Ethanol/Sodium Acetate and dissolved in 10 μl HiDi formamide. The resulting fragments were run on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The sequences were aligned by eye using Bio Edit 7.0.5.3 (Hall 1999), resulting in sequences of 850 base pairs.

Five microsatellite loci (HG14, HG16, HG18, HG25 and HG27), developed for the North American herring gull *L. (a.) smithsonianus* were used, following the procedures in Crochet *et al.* (2003). PCR was performed in a 15 μl reaction containing the same concentrations as listed above. Microsatellites were amplified with fluorescently labelled forward primers (Applied Biosystem), samples were sent for genotyping to GATC Biotech AG, Germany and scored with the Gene Marker 5.1 software package (SoftGenetics LLC 2004).

Mitochondrial data. Basic statistics of mtDNA diversity, including nucleotide and haplotype diversity for each sample, deviation from non-equilibrium dynamics, and population structure were estimated with Arlequin 3 (Excoffier and Schneider in press). In order to detect deviations from equilibrium population dynamics, resulting from population expansion or admixture, pairwise differences among all sequences were calculated and mismatch distributions were drawn (Rogers and Harpending 1992). Tajima's D (Tajima 1989) was

calculated to estimate the impact of selection or non-equilibrium dynamics on nucleotide diversity and number of segregating sites. To detect signatures of population structure, hierarchical analyzes of molecular variance (Amova) was calculated among species and populations (Excoffier *et al.* 1992) and the proportion of variation (F_{st}) for all pairwise comparisons between samples were calculated, based on haplotype frequencies and on variance in number of mutations among haplotypes (Weir and Cockerham 1984). For comparison, the proportion of variation based on variance in allele size (R_{st}) was calculated as well (Slatkin 1995). The overall relationship among the haplotypes found is presented with a median-joining network (Bandelt *et al.* 1999) constructed with Network 4.201 (www.fluxus-engineering.com/sharenet.htm). Equal weights were assumed for each variable position.

Microsatellite analyzes. Genetic diversity parameters; number of alleles (*na*) and unbiased expected heterozygosity (*He*), were summarized with Genetix v. 4.05 (Belkhir *et al.* 2004). Effective number of alleles was calculated as *ne* = 1/(1-*He*). Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested with random permutations as implemented with Genetix. Hierarchical partition of variation among species and population were conducted with Arlequin 3 (Exoffier and Schneider, in press). Pairwise F_{st} values (Weir and Cockerham 1984) and Nei unbiased genetic distance (Nei 1978) were calculated with Genetix 4.03 (Belkhir *et al.* 2004). Genetic distances based on proportions of shared alleles were calculated, taking into account only private alleles with frequencies above 10% within samples. The pairwise differences were summarized with a multidimensional scale plot, also known as principal coordinate analyzis (Venables and Ripley 1994). The genetic structure, including the mtDNA haplotypes was also analyzed by the Bayesian clustering method and the admixture analyzes implemented in BAPS 4.14 (Marttinen *et al.* 2006). The analysis was based on samples which had been scored for three or more markers. In BAPS the a priori information of the 10 samples listed in Table 2 was used for clustering. Evidence for admixture was considered significant for individuals with a Bayesian *P*-value < 0.05. A neighbour joining tree of the distances between the clusters obtained by BAPS was calculated using PHYLIP (Felsenstein 1993).

Results

Analysis of the mitochondrial cytochrome b

In the 850-bp section of the cytochrome *b*, 41 polymorphic sites were identified in 218 gulls analyzed. All variation was in the form of nucleotide substitution. Altogether, 21 haplotypes were observed and seven of them were shared between species (Table 2).

Table 2: mtDNA haplotypes, nucleotide diversity and haplotype diversity for each sample at a given time period in all locations. Population names correspond to Table 1.

Populations	n	mtDNA haplotypes	Nucletide diversity x 100	Haplotype diversity
A1A	29	A12, A25, A26, A6, Ma, Ma2, H2	0.1826	0.6897
A2A	26	A12, A14, A20, A23, A27, A6, Ma, Mi3	0.4463	0.8300
A3A	24	A12, A20, A23, A24, A6, Ma	0.3733	0.7933
A3V	7	A12, A21, A6, H2	0.3810	0.8095
H1V	27	A12, A20, H2, H8	0.1877	0.5755
H2V	22	A12, H2	0.0535	0.4546
H3V	56	A12, A22, H1, H2, H6	0.0848	0.5571
H1A	2	Ma, A12	0.1176	1.0000
H3K	18	G1, A12, H1, H2, H7, G3	0.2853	0.7974
G	7	G1, H1	0.0392	0.3333
	218			

Haplotypes differed from each other by a maximum of 10 mutations (Figure 2). In the median-joining network analysis, 137 individuals from Liebers *et al.* (2004) and Crochet *et al.* (2003) were included and 17 new haplotypes were added (and 5 shared), adding a more detailed geographic view of the network. The 38 haplotypes form two main clades: a North American clade and a European clade (Figure 2).

The American clade includes 3 haplotypes (Ar1, Ar2, Ar3) from the American herring gull *L.(a.) smithsonianus* (Alaska and Canada), 6 haplotypes (Ar10, Hy1, Hy7, GL1, GL2, GL3) from *hyperboreus* (NE-Canada and Kulusuk) and *glaucoides* (NE-Canada, Kulusuk and Iceland). In Kulusuk, *hyperboreus* held six haplotypes, three of them were not found in Icelandic conspecifics. The seven *glaucoides* studied held two haplotypes and one of them was only found in *glaucoides*. One Icelandic adult *hyperboreus* (from Grundafjordur in west Iceland) had the Hy1 haplotype and was grouped with the *hyperboreus* from Kulusuk. This was the only European gull that had a North American haplotype.

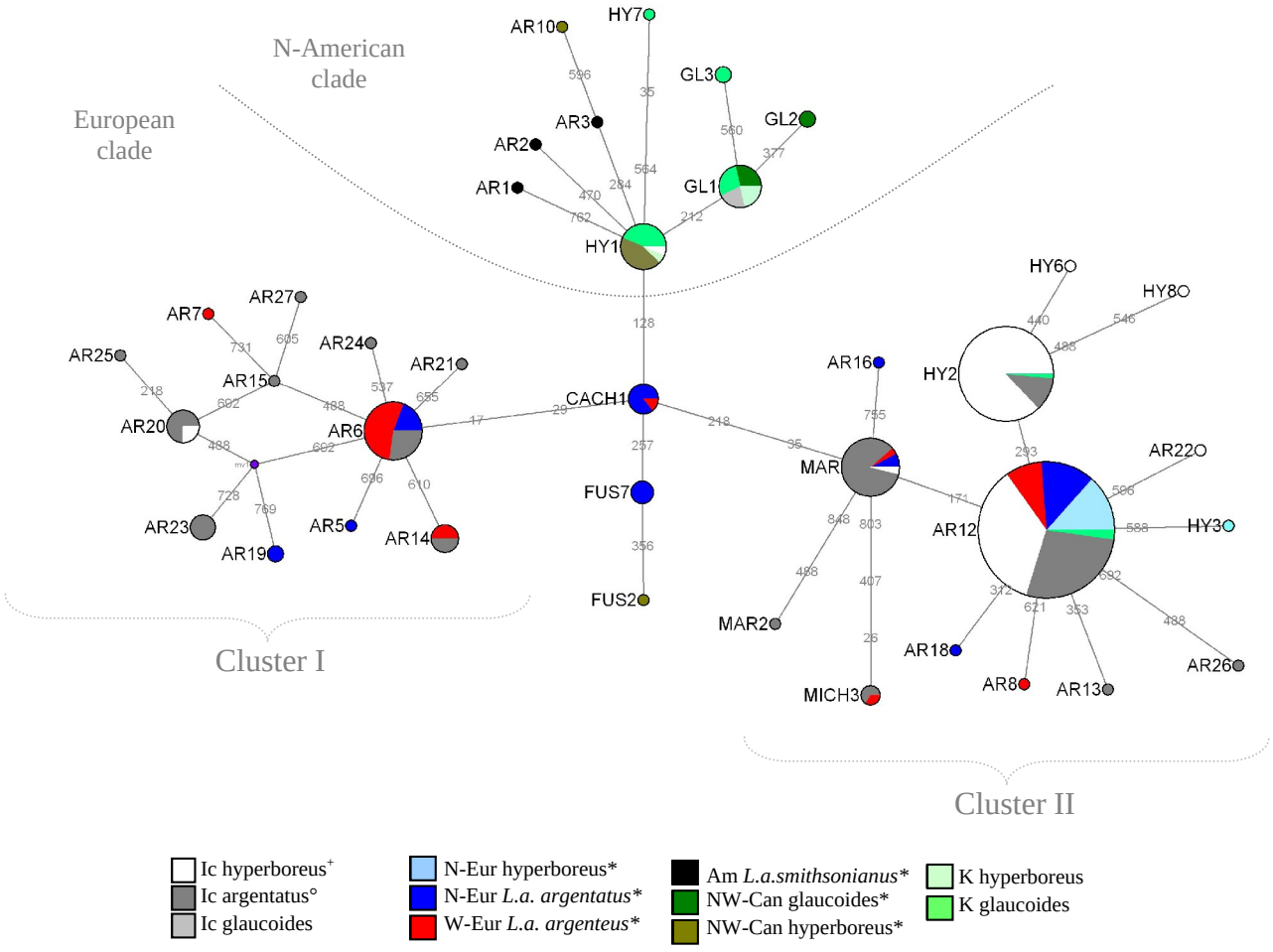

Figure 2. Haplotype network of the 38 haplotypes from cytochrome *b* from Iceland (Ic), Kulusuk in Greenland (K), North America (Am), North-East Canada (NE-Can), North Europe (N-Eur), West Europe (W-Eur). Circles represent unique haplotypes and the size reflects the number of individuals sharing a particular haplotype. Colours refer to different geographic regions and species. Different clades and clusters are indicated.
*Data from Liebers et al (2004), +10/114 specimens from Libers et al (2004), °15/100 specimens from Liebers et al (2004)

The European clade constitutes 29 haplotypes, 4 of these are shared between *hyperboreus* and *argentatus* (Ar20, Mar, Ar12, Hy2) and the other 4 (Ar6, Ar14, Cach1, Mich3) are shared between the conspecifics *L.a. argentatus* and *L.a. argenteus*. The European clade is divided into two clusters I and II, connected through the Cach1 and Fus haplotypes which were previously described for *L. cachinnans* and *L. fuscus* (Liebers *et al.* 2004). These haplotypes were found in N- and W-European *argentatus*, Fus2 was also found in one *hyperboreus* from Baffin Island (Liebers *et al.* 2004; Crochet *et al.* 2003).

Icelandic birds classified as *hyperboreus* held 8 haplotypes, 3 unique (Hy8, Hy6, Ar22) and 4 shared (Ar20, Mar, Ar12, Hy2) with *argentatus* and one (Hy1) with conspecifics from Kulusuk and Canada. Only one haplotype (Ar12) was shared between Icelandic and

European *hyperboreus.* Icelandic *argentatus* held 16 haplotypes in addition to the 4 shared with *hyperboreus,* they shared 3 of them (Ar6, Ar14, Mich3) with *argentatus* from N- and W-Europe and had 9 haplotypes found only in Iceland (Figure 2).

Cluster I is formed by 54 individuals and 12 rather ramificated haplotypes. Only two individuals were *hyperboreus* but otherwise the ramification is characterized by *argentatus* individuals. The most widespread haplotype was Ar6, found in 26 individuals, representing half of the individuals in cluster I. About 80% individuals were from Iceland and W-Europe (France, Netherlands and Faroe Islands), representing the *L.a. argenteus* group (Barth 1968). The six individuals representing the *L.a. argentatus* group, came from more southern range of the groups' distribution, Germany, Finland and Estonia. Icelandic individuals in cluster I were represented by 26 *argentatus* whose proportions increased significantly from time period 1 (7%) to periods 2 and 3 (42% and 32%) ($P < 0.0039$, Fisher exact test). Additionally, 2 *hyperboreus* from west Iceland from period 1 were also clustered here.

Cluster II holds 14 haplotypes among 249 individuals, 138 *hyperboreus* and 111 *argentatus*. Most individuals in this cluster originate from northerly areas, where the distribution of the two species is adjacent or overlapping. The majority are represented by haplotype Ar12 (59%) or the derivative Hy2 (29%).

The ratio of *hyperboreus* in cluster II increases when following the lineages from Mar to Ar12 and becomes predominant in Hy2 and derived haplotypes. The Hy2 group is represented by 99% Icelandic originates, 60 *hyperboreus* from west Iceland, and 8 *argentatus* from west (period 3) and southeast (period 1) Iceland. No other *argentatus* were found in such "*hyperboreus*-like" group.

As well as being the most prevalent, Ar12 was also the most geographically variable haplotype. Among 74 *hyperboreus* it was found in all Icelandic sampling locations (though mostly in west Iceland), Kulusuk and in N-Europe (Svalbard and Novaja Semlja (Liebers *et al.* 2004). Of the 73 *argentatus* with Ar12, 39 originated from eastern Iceland, 18 from North Europe and 12 from W-Europe (Liebers *et al.* 2004). The only cases of American originates found in the European cluster were 4 *hyperboreus* from Kulusuk, 3 Ar12 and 1 Hy2. The Mar haplotype was found in 22 Icelandic *argentatus*, 1 W-Europe (Faroe Islands) *L.a. argenteus* and 2 N-European (Finland and Estonia) *L.a. argentatus* (Liebers *et al.* 2004). One Icelandic *hyperboreus* (Hromundarey) also shared the type with this otherwise "*argentatus*-like" group. The Mar haplotype has only been detected in Icelandic *hyperboreus.* About 40% of Icelandic *argentatus* in cluster II were from time period 1, 28% from period 2 and 32% from period 3.

Hyperboreus grouped biphyletically in both clusters, but was much more prevalent in cluster II which is represented by *hyperboreus* and *argentatus* from northerly latitudes, where the distribution of the two species comes to its closest. *Argentatus* seems to be monophyletic in the European clade, but with separate centres in clusters I and II. Cluster I represents *argentatus* from Iceland and more southern European individuals.

Excluding H1A because of low sample size, the overall nucleotide and haplotype diversity was higher in *argentatus* groups compared to *hyperboreus.* Interestingly, *hyperboreus* from Kulusuk were more divergent than all *hyperboreus* groups from Iceland. Haplotype diversity was generally stable over time among the species. In *argentatus,* nucleotide diversity increased through time from (0.18% to 0.45% and 0.37% in the consecutive periods. In *hyperboreus,* nucleotide diversity decreased through time, from 0.19% to 0.06% and 0.08% (Table 2). The high diversity during the first period likely results from the *argentatus* haplotype Ar20, present in 2 *hyperboreus* individuals.

The mismatch distributions reflected the observed difference in the diversity of the *hyperboreus* and *argentatus* haplotypes. A bimodal distribution was observed in *argentatus,* but *hyperboreus* showed a unimodal distribution **(**Figure 3). Tajima's *D* was insignificant in all cases, indicating neutrality of the observed variation.

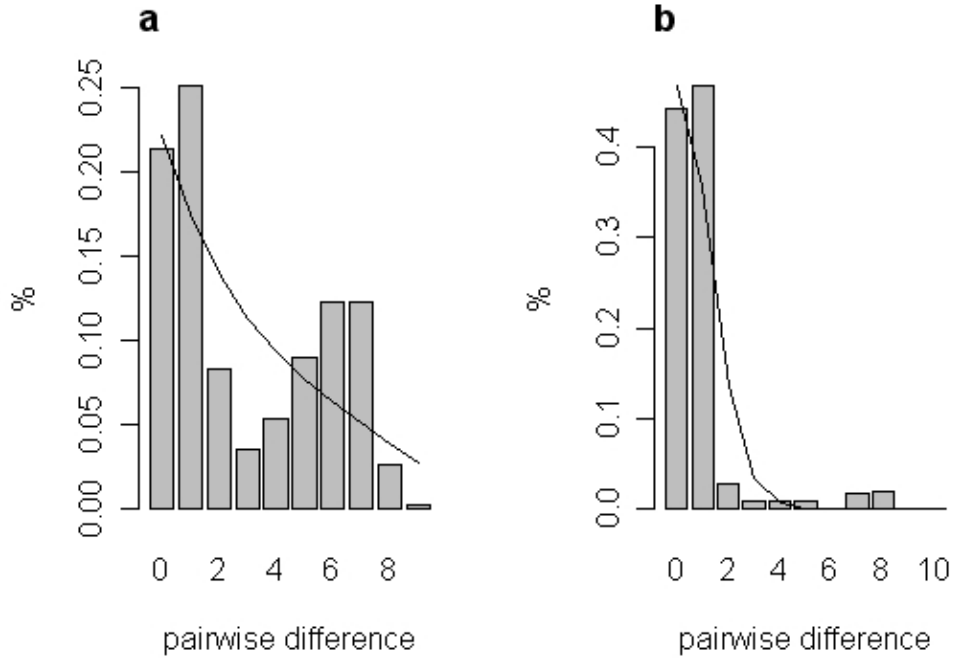

Figure 3. Frequencies of pairwise sequence differences between haplotypes (mismatch distributions) within *argentatus* (a) and *hyperboreus* (b). On the horizontal axis, i is the number of nucleotide site differences between pairs of individuals. The histogram shows the relative frequencies of pairs with i differences. The solid line is the theoretical mismatch distribution.

Microsatellite analysis

Details of the genetic diversity, numbers, effective numbers, and frequencies of alleles in the 10 samples are given in Table 3.

Table 3. Allele frequencies for the five microsatellite loci in 10 samples (correspond to Table 1).

Loci			A1A	A2A	A3A	A3V	H1A	H1V	H2V	H3V	H3K	G
HG 14	Allele size range	116-134										
	No of alleles	9	4	6	4	6	2	5	6	5	4	5
	H exp		0.4211	0.4759	0.4471	0.7578	0.2449	0.5828	0.6056	0.5974	0.4956	0.74
	Fis W&C		0.134	0.114	-0.048	0.077	-0.091	0.316	-0.036	-0.024	0.226	0.294
	P		10.5	21.5	52	46.9	92.4	0.1**	51.9	44.7	17.7	16.1
HG 18	Allele size range	108-125										
	No of alleles	9	6	7	5	4	4	7	6	4	4	6
	H exp		0.6684	0.7029	0.6881	0.6484	0.5781	0.6703	0.6619	0.561	0.6259	0.7755
	Fis		0.15	0.039	0.137	0.103	-0.014	-0.023	0.234	-0.056	-0.041	-0.029
	P		5.6	42.3	21.2	48.7	63.1	46.1	4.1	33.5	52.6	69.6
HG 25	Allele size range	109-126										
	No of alleles	11	8	8	6	6	5	8	7	6	5	6
	H exp		0.7631	0.8331	0.7913	0.6875	0.6641	0.6892	0.772	0.5887	0.4342	0.7755
	Fis		0.384	0.088	0.332	0.506	-0.063	0.284	0.191	0.698	0.037	0.155
	P		0***	16.8	0.4**	0.6*	56	0.1**	5.5	0***	54.1	32.4
HG 27	Allele size range	114-116										
	No of alleles	3	3	3	2	3	3	3	3	3	3	3
	H exp		0.3297	0.429	0.4537	0.6328	0.3984	0.2018	0.2908	0.3904	0.5159	0.5204
	Fis		-0.019	-0.319	-0.517	0.273	-0.191	-0.08	0.161	-0.126	0.556	-0.304
	P		66.5	3.2	2.5*ath -	23.2	59.9	53.8	29.9	22.4	0.1**	30.8
HG 16	Allele size range	170-187										
	No of alleles	8	2	4	3	2	1	3	3	2	4	4
	H exp		0.0571	0.3314	0.3089	0.1172	0	0.4063	0.455	0.4786	0.4474	0.4592
	Fis		0	-0.121	0.005	0	------	0.44	0.38	0	-0.032	0.442
	P		100	62.4	52	100	NA	13.4	9.5	62.4	59.5	9.7
All loci	*N*	310	63	37	23	8	8	53	27	63	21	7
	na	8	4.6	5.6	4	4.2	3	5.2	5	4	4	4.8
	ne 1-(1-He)		1.8	2.2	2.2	2.3	1.6	2.0	2.3	2.1	2.0	2.9
	He		0.448	0.554	0.538	0.569	0.377	0.510	0.557	0.523	0.504	0.654
	Hobs		0.363	0.568	0.529	0.475	0.432	0.413	0.469	0.464	0.441	0.634
	Fis		0.1979***	-0.0079	0.0399	0.229*	-0.0788	0.2102**	0.1803**	0.1217*	0.15022*	0.1161

N, number of successfully analyzed individuals; na, average number of observed alleles; ne, effective number of alleles; He, expected average heterozygosity; Hobs, observed average heterozygosity; Fis, proportion of variation (Weir & Cockerham 1984) ***$P \leq 0.001$, **$0.001 < P \leq 0.01$, $0.01 <$ *$P \leq 0.05$, underlined *P*-values denote significant values after Bonferroni correction.

The effective number of alleles was highest (2.9) in *glaucoides*, possibly reflecting the diverse origin of individuals in that sample. In the *argentatus* populations this value ranged from 1.8 to 2.3 and the value in *hyperboreus* ranged from 1.6 to 2.3. The lowest value was observed in H1A. The total number of alleles per locus ranged from 3 to 11, with all populations combined. Allele distribution and frequencies were variable between loci and species. Species-specific allele (diagnostic allele) was detected in high frequencies among *hyperboreus* in locus HG16 and seems characteristic for the respective species (Figure 4).

Proportions with respect to average expected heterozygosity were similar, H1A had the lowest value (0.38) and *glaucoides* had the highest (0.65). The most variable loci were HG25 (He = 0.70) and HG18 (He = 0.66), while the least variable locus was HG16 (He = 0.31). Differences in alleles between populations were mainly presented by frequency

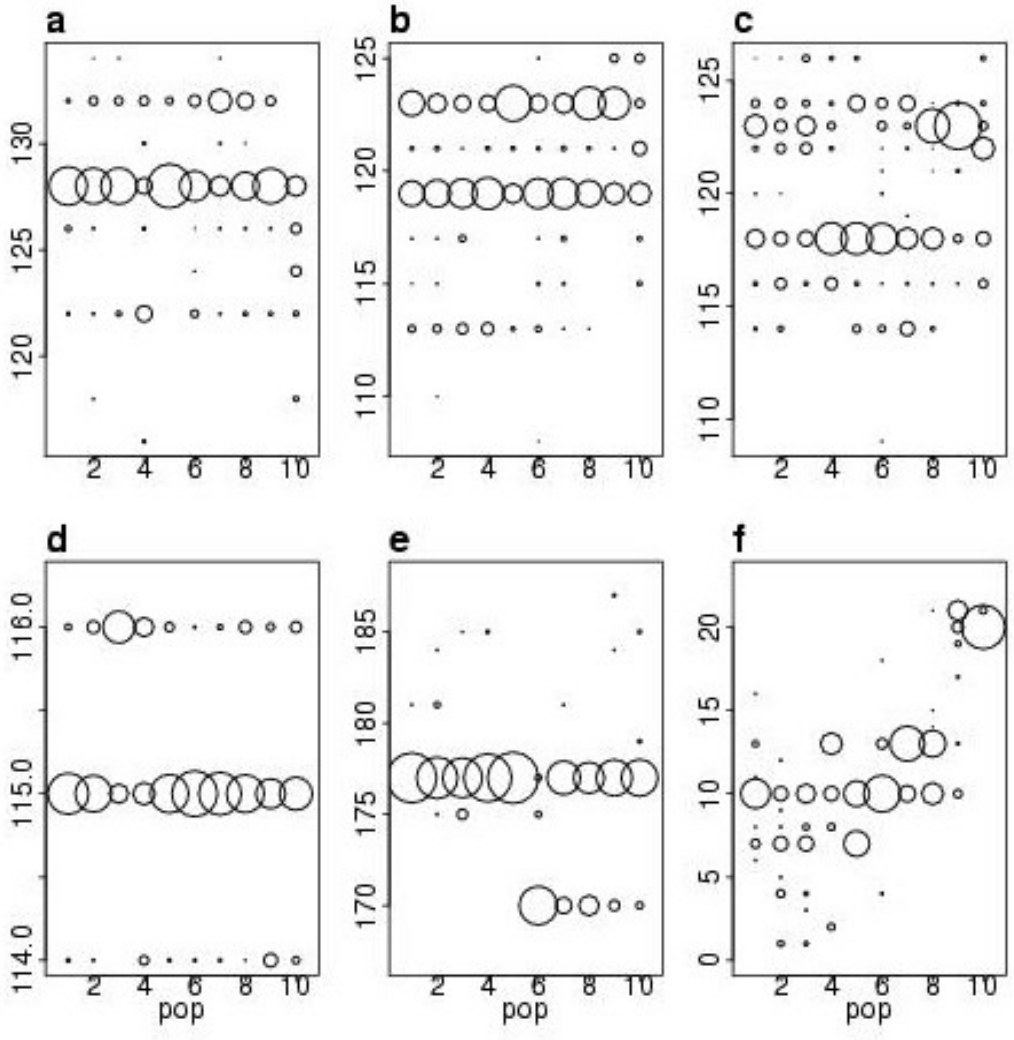

Figure 4. Microsatellite allele distributions. Each circle shows one allele and its size represents the frequency in the respective population, 1-10 (correspond to Table 1).

difference and not many low frequency alleles were detected. This is seen by the small difference between the average absolute number of alleles across loci (3 - 5.6) and the effective number of alleles (1.6 - 2.9). In Iceland, A3V displayed the largest He (0.569) and ne (2.3). Populations from era 1, A1A and H1A, had lower genetic variability as reflected by He (0.448 and 0.377) and ne (1.8 and 1.6). F_{is} was only significant in three populations after Bonferroni correction, in A1A, H1V and H2V (Table 3). Locus HG25 displayed the most significant F_{is} (after Bonferroni correction), presented in three populations; A1A, H1V and H3V. Alleles displayed no tendency of change through time and He across loci was not significantly correlated to eras (Wilcoxon test: $P < 0.05$) in *argentatus* and *hyperboreus*. Seven out of 100 tests of linkage disequilibrium gave permutation values larger then or equal to observed *P*-values in the range of 0.8% to 3.1%. This weak signal of linkage disequilibrium can be explained by coincidence.

Population genetic differentiation

Hierarchical analysis of the genetic variance shows a significant differentiation among and within population. Differentiation among species was in all cases non-significant representing an intra-specific variance. The mtDNA was close to significance (p = 0.066 and 0.0576) but the microsatellites were highly non-significant (p = 0.551) **(**Table 4).

Table 4. Hierarchical analysis of molecular variance (AMOVA) of mtDNA and microsatellite variation among *argentatus* and *hyperboreus* in Iceland. *P*-values are based on 1023 permutations of the data, which resulted in equal or larger value than the observed statistic.

Molecular marker /method	Soruce of variation	Total variance (%)	Fixation indices	*P* value
mtDNA	Among groups	13.88	*F*ct = 0.139	ns
Conventional F-stats	Among populations within groups	3.342	*F*sc = 0.042	< 0.05
	Within populations	82.55	*F*st = 0.175	< 0.001
mtDNA	Among groups	24.7	*F*ct = 0.247	ns
Pairwise difference	Among populations within groups	7.37	*F*sc = 0.098	< 0.01
	Within populations	67.93	*F*st = 0.321	< 0.001
Microsatellites	Among groups	-1	*F*ct = -.0.0099	ns
	Among populations within groups	10.54	*F*sc = 0.1044	< 0.001
	Within populations	25.61	*F*st = 0.283	< 0.001

The extent of differentiation among population within species is larger when variance in genetic differentiation among haplotypes is taken into account (increases from 3.4% to 7.4%). Similarly, calculations of R_{st} revealed similar results (data not shown) except a substantially higher total variance among population within groups (40.21%). A closer inspection of where the deviation occurs among samples is presented in Table 5. The species were, overall, significantly different from each other. *Glaucoides* (G) differed significantly from all other population group with conventional F_{st}, but became non-significantly different from H3K using pairwise F_{st}. *Hyperboreus* from Kulusuk also differed from all groups except H1A, which was presented by only 2 individuals with haplotypes so it has no validity in further mtDNA results. Icelandic *hyperboreus* populations differ from all but one *argentatus* population, A3V. Additionally, H1V was not significantly different from A1A, which was mainly represented by individuals from Hromundarey. Interestingly, H1V is significantly different from H2V (P< 0.05) and becomes significantly different (P< 0.05) from H3V with

pairwise F_{st}. Within *argentatus* populations, A1A was different from A2A ($P < 0.05$) and A3V was different from A2A with conventional F_{st} ($P < 0.05$), but was non-significant with pairwise F_{st}. F_{st} values for mtDNA were more significant when using pairwise differences methods than conventional F_{st}.

Table 5. F_{st} values among populations: mtDNA (below diagonal) and microsatellites (above diagonal). Number of stars denote significance (***$P \leq 0.001$, **$0.001 < P \leq 0.01$, $0.01 <$ *$P \leq 0.05$).

Samples	A1A	A2A	A3A	A3V	H1A	H1V	H2V	H3V	H3K	G
A1A	-	0.01424*	0.09905***	0.08642**	0.01478	0.23042***	0.08864***	0.07959***	0.06958***	0.06402*
A2A	0.0461*	-	0.04898***	0.04733*	0.0135	0.18067***	0.05044***	0.07748***	0.08148***	0.02124
A3A	0.0141	-0.0228	-	0.04787*	0.0951**	0.25531***	0.13933***	0.12209***	0.12376***	0.07421**
A3V	0.0599	0.1016*	0.0907	-	0.07333*	0.22856***	0.07413*	0.11173***	0.13295**	0.0085
H1A	-0.2326	-0.2262	-0.2636	-0.0106	-	0.20018**	0.0494*	0.0964**	0.12042***	0.06864*
H1V	0.0304	0.1466***	0.1244**	0.0373	0.0293	-	0.09451**	0.10654***	0.19462***	0.15655**
H2V	0.2219*	0.2901***	0.2909***	0.0052	0.3366	0.1526*	-	0.05439***	0.11187***	0.05477*
H3V	0.1233**	0.2284***	0.2159***	-0.0205	0.1701	0.0449	0.0062	-	0.03159**	0.09501**
H3K	0.1832***	0.1458***	0.1459***	0.1357*	0.0749	0.2321***	0.3191***	0.2640***	-	0.09417***
G	0.4221***	0.3414***	0.3433***	0.4160***	0.5102***	0.4967***	0.5812***	0.4983***	0.1856*	-

In five cases significant comparisons became insignificant, and in other five cases the significance level was reduced when using conventional F_{st} instead of pairwise differences. This was represented between comparisons of conspecifics at different eras (H3V and H2V versus H1V and H1A; A3A and A2A versus A1A) but also different species at the same locations (A3V versus H3V and H2V). Estimates of genetic differentiation between all ten groups, using F-statistics, were more often significant for microsatellites (41/45) than for cytochrome *b* (27/45) (Table 5). But it has to be noted that aside from more markers being used, 93 more individuals were successfully analyzed with microsatellites compared to mtDNA. The g*laucoide*s group and H3K showed the highest deviation values compared with the remaining populations (maximum value of 0.5812 between G and H2V) in mtDNA. In the microsatellites the only comparisons showing more than one instance of any insignificance were H1A, A2A and G. All other pairings were highly significant (maximum value of 0.25531 between A3A and H1V).

The differentiation of the groups, by distances based on shared alleles, is presented in the multidimensional scale plot (Figure 5). Well defined grouping can be identified separating *argentatus* and *hyperboreus* in time and space. The distinctive cluster representing *argentatus* samples, also includes *hyperboreus* sampled in the first time period in southeast Iceland (H1A). *Hyperboreus* from Greenland is clearly distinct from the Icelandic *hyperboreus.* Scaleplot based on Nei distances and F_{st} values gave similar results (data not shown). Interestingly, H1A held its position in the *argentatus* cluster with all methods.

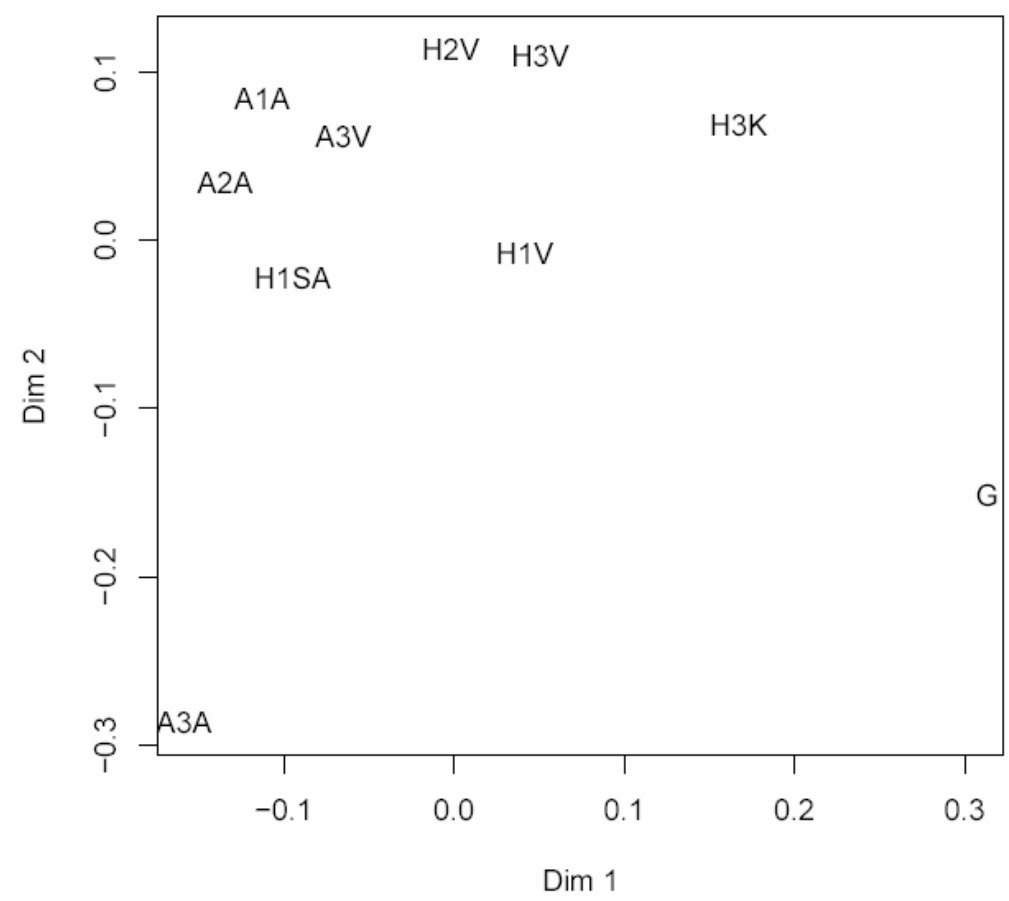

Figure 5. Multidimensional scaleplot based shared alleles (b). Samples correspond to Table 1.

Based on both the mtDNA and the microsatellite data, the Bayesian clustering method (BAPS) gave an optimal partition of 5 clusters with propability of 99,9%. The log likelihood for different numbers of clusters k from 2-5 were: -3747.1, -3673.6, -3656.5 and -3655.3. Larger numbers of initial groups (6-8) resulted in 5 clusters. A non-signifciant difference was between four and five clusters, reflecting the fact that there is no statistical support for the

distinction between the *glaucoides* and the *hyperboreus* from Kulusuk. All *argentatus* groups were clustered together in cluster 1, plus the H1A group. All *hyperboreus* from the west of Iceland were in cluster 2 (H1V, H2V) and 3 (H3V) and were equally distant from cluster 1. *Hyperboreus* from Kulusuk (Cluster 4) clusters further apart from the previously mentioned clusters and the *glaucoides* (Cluster 5) are clustered even further apart (Figure 6).

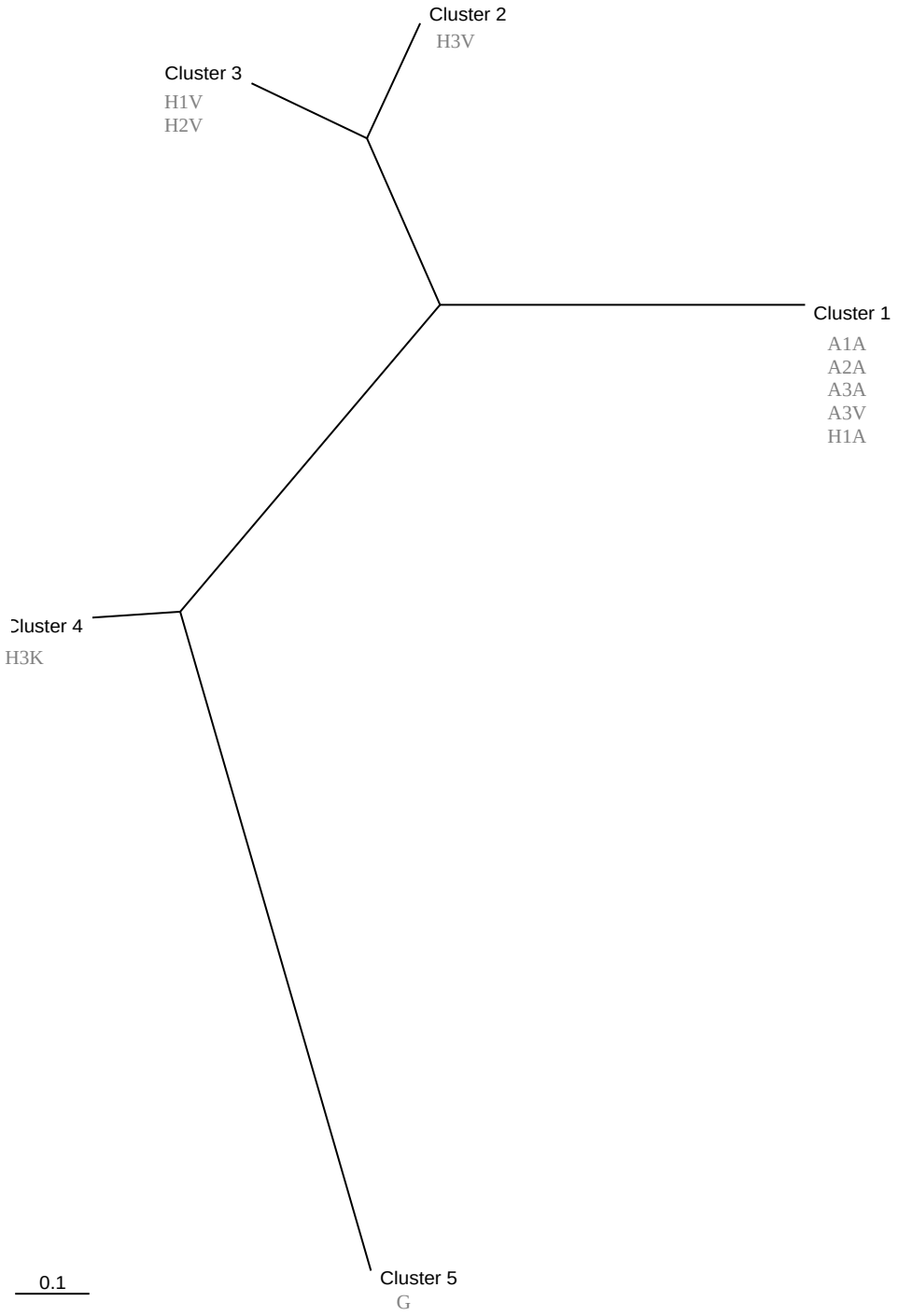

Figure 6. Clustering based on results of group level mixture analysis by BAPS (Marttinen *et al.* 2006).

The proportion of the clusters that were more likely to belong to another cluster is presented in Figure 7. 36 out of 142 individuals (25%) in cluster 1 were assigned to another cluster. Four of these were *hyperboreus* from H1A. Close to 9% of the *hyperboreus* individuals in cluster 2 (H1, H2) were more likely to be assigned to another cluster, 5 individuals (6%) were most likely to belong to Cluster 3 (H3). A larger fraction of cluster 3 was more likely to be assigned to another cluster, 7 individuals (or 12%) were assigned to cluster 2, showing the close relationship of the *hyperboreus* samples. No individuals from cluster 2 and 3 were assigned with the *argentatus* in cluster 1, two were assigned with the *hyperboreus* from Kulusuk. Overall, a direction in the introgression from h*yperboreus* to *argentatus* is observed. Only the 4 *hyperboreus* individuals from H1A which clustered in cluster 1 were assigned with the *argentatus* genotypes. Thus, 4 out of 149 *hyperboreus* individuals were assigned to cluster 1. From cluster 1, 32 out of 134 *argentatus* individuals were assigned with the *hyperboreus* in clusters 2, 3 and 4. 22 of these 32 individuals were sampled in time period 1 in southeast Iceland. The association is significant (P = 2.2e-16, Fisher´s exact test). In the Kulusuk sample, 2 out of 19 were assigned to cluster 3 (H3). Although cluster 5 was not statistically significant from cluster 4, only *glaucoides* individuals were assigned to this cluster.

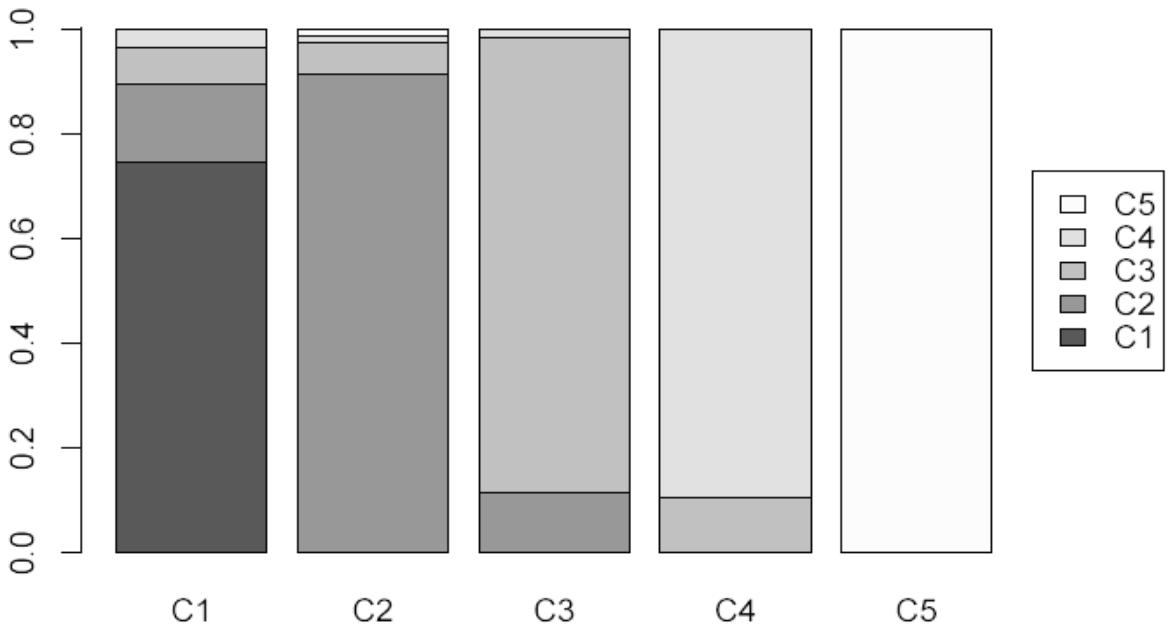

Figure 7. Proportions of individuals in each cluster (C1-C5) assigned to another cluster. The admixture analysis is based on the results from BAPS (Marttinen *et al.* 2006). Samples in clusters correspond to figure 6.

Discussion

Phylogeography

The herring gull, *Larus argentatus*, and the glaucous gull, *Larus hyperboreus*, both have a geographically wide distribution throughout the northern hemisphere with the latter confined to arctic or subarctic regions. A previous study of mtDNA phylogeography by Liebers *et al.* (2004) has shown that the two species do not correspond strictly to distinct monophyletic mtDNA haplogroups. Both species appear biphyletically in the American and the European clades, and very limited haplotype sharing is detected between the two clades. In the European clade, *argentatus* presents a large ramificated tree consisting of two distinct clusters (I and II), separated by haplotypes which are shared by other gull species, namely *L. cachinnans, L. fuscus* and *L. marinus* (Liebers *et al.* 2004). *Hyperboreus* is represented biphyletically in the European clade, appearing in the two European *argentatus* clusters, although mainly in cluster II. This lack of monophyly of *hyperboreus* in Europe could be due to incomplete sorting of mtDNA lineages from a polymorphic ancestral gene pool but more likely due to hybridization, as discussed below.

The mtDNA of *hyperboreus* is characterized by low diversity, as has been observed for several Arctic species (Hewitt 2001). The low diversity and shape of the phylogenetic network may reflect fluctuations in population size (Hein *et al.* 2005) which may have been more severe for the mtDNA than the nuclear loci due to smaller effective population size. The Icelandic *hyperboreus* population belongs to the European *hyperboreus* clade, but is clearly distinct from the populations sampled elsewhere. Only one *hyperboreus* out of 117 sampled in Iceland and an additional 20 from Svalbard and Novaja Zemlja shared an "American" haplotype (H1) with *hyperboreus* from Kulusuk and Baffinland, NE-Canada (Liebers *et al.* 2004). This infers little contact between the Icelandic and the East-Greenland population reflecting the division between the Palaearctic and the Nearctic *hyperboreus*. This division was also supported by the microsatellite data.

Overall, *argentatus* harbour larger number of haplotypes and higher diversity in mtDNA in Iceland compared to *hyperboreus*, in agreement with the previous genetic studies of *argentatus* in Europe (Crochet *et al.* 2002, 2003; Liebers *et al.* 2004). The Icelandic population differs from the rest of Europe, showing greatest similarity to the samples from northern Europe. Interestingly, comparisons with other samples from Europe, obtained by Liebers *et al.* (2004), show that the earliest *argentatus* samples were most similar to *hyperboreus* samples and northern populations, in Norway and Russia (Figure 8).

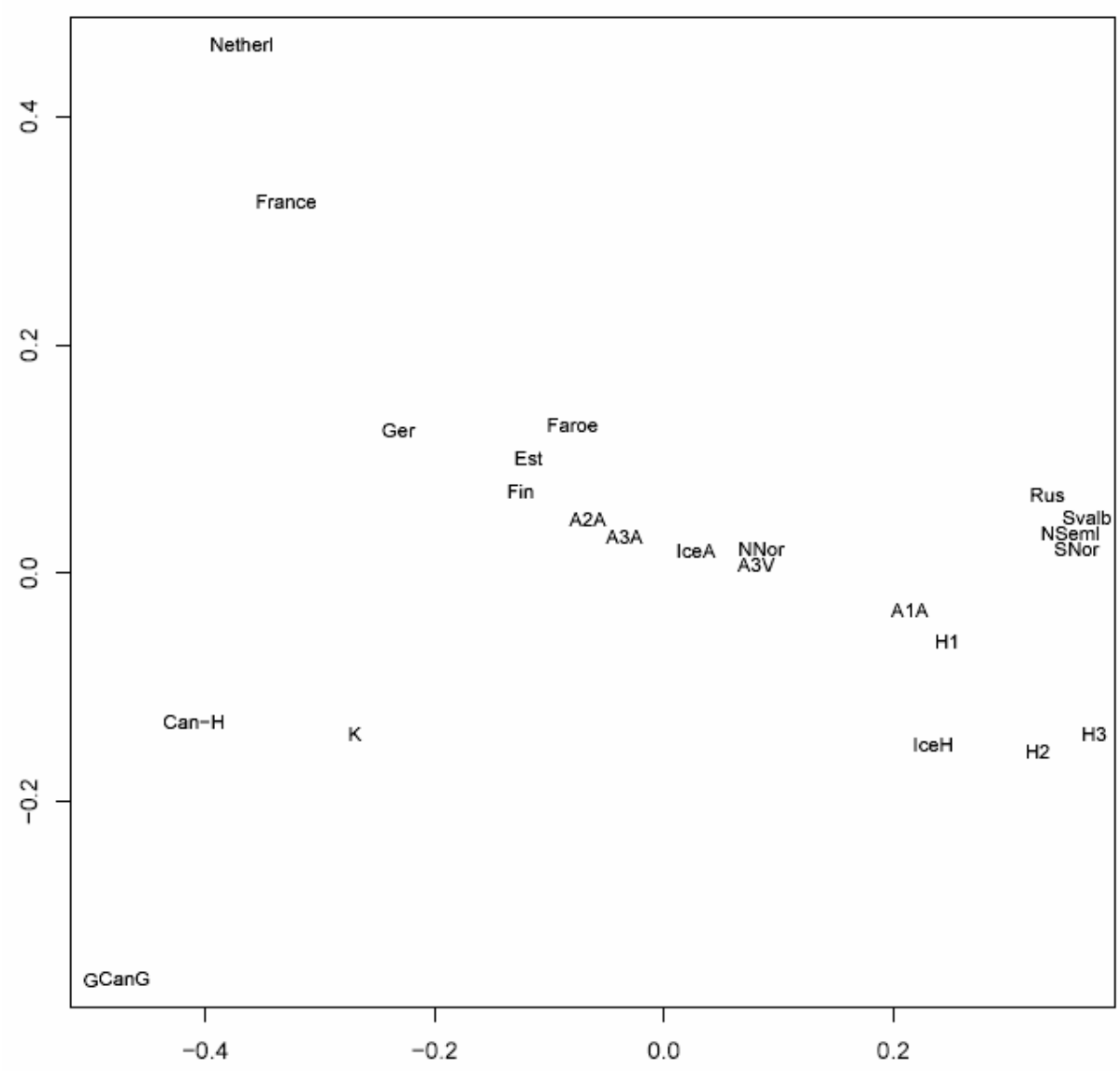

Figure 8. Multidimensional scaleplot based on mtDNA data from this study[+] and from Liebers *et al.* (2004*). [+]A2A, A3A, A1A, H1, H2, H3, G: Correspond to Table 1. K: Kulusuk *hyperboreus.*

**Argentatus*; Netherl: Netherlands, France: France, Ger: Germany, Faroe: Faroe Islands, Est: Estonia, Fin: Finland, SNor: Sourth Norway, NNor: North Norway, Rus: Russia, IceA: Icelandic *argentatus*. *Hyperboreus*; Svalb: Svalbard, NSeml: Novaja Semlja, IceH: Iceland, Can-H: Canada Baffinland. *Glaucoides*; CanG: Canada Baffin Land.

On the other hand, *argentatus* from time periods 2 and 3 were more similar to populations with more southern and western distribution, sharing haplotypes with individuals from e.g. France, Faroe Islands and Germany. A closer look at the composition of the haplotypes in Icelandic *argentatus* showed that the proportion of the cluster I and II changed over time. Cluster II was dominant during the first time period (92%), whereas haplotypes in cluster I increased in frequency with time, up to 40% in 1985-1986. This change is also reflected in the

nucleotide and haplotype diversity, and may reflect later waves of colonization or immigration of *argentatus* to Iceland. This may also explain the bimodal mismatch distribution in *argentatus.* Bimodality is generally interpreted as a sign of stable evolutionary population size, but here it may also arise due to an admixture of *argentatus* populations. An alternative hypothesis is that the bimodality reflects hybridization over a long period, where cluster I has spread from *hyperboreus* to *argentatus* by introgression.

Hybridization

Another characteristic result of the *hyperboreus* mtDNA is a lack of distinct haplotypes, which are not shared by *argentatus*. Most of the haplotypes are found in cluster II and only three of them have not been found in *argentatu*s. Furthermore these unique haplotypes are rare, found only in single individuals. The most common haplotype (A12) is shared by *argentatus* in northern and northwestern Europe and is the only haplotype shared between *hyperboreus* and *argentatus* outside Iceland. It is tempting to jump to the conclusion of shared ancestry rather than introgression, but when looking at places of origin, most *argentatus* individuals found with this haplotype were sampled where the distribution zones of the two species are close or overlapping, faciliating introgression.

However, sharing of other haplotypes among the species is found only in Iceland. One common haplotype which is not found elsewhere in Europe is common both in Icelandic *argentatus* and Icelandic *hyperboreus*. As *argentatus* is a recent settler in Iceland this suggests recent introgression rather than shared ancestry. Three instances are observed where widely distributed *argentatus* haplotypes (Mar and A20) are found in *hyperboreus*, only in Iceland, in samples from the first time period (1964-1973). One of these haplotype is from cluster I (A20), and it caused the high nucleotide diversity seen in *hyperboreus* during the first time period. This unique finding of haplotype sharing where *hyperboreus* and *argentatus* co-occur, indicates introgression. If shared ancestry were the case, these haplotypes would most likely have been found in other *hyperboreus* elsewhere. Less sharing of the haplotypes in cluster I among the two species may be expected, as the *argentatus* with haplotypes found in cluster I appear to have arrived to Iceland more recently.

The lack of significance within the species in the hierarchical analysis, points to a high intra-specific variability, possibly due gene flow between the species. The finding of introgression is supported by microsatellite data as well. The lack of significant difference between sympatric groups of the two species was clearly seen in all pairwise comparisons based on microsatellites, implying gene flow**.** Bayesian clustering analysis (BAPS) based on

mtDNA and microsatellite results gave similar findings where a *hyperboreus* group (H1A) sharing the *argentatus* types, clustered among the *argentatus.* Significant F_{st}'s were observed for microsatellites between groups that were sharing mtDNA haplotypes, indicating that these populations hold individuals that belong to two groups. Supporting these signs of hybridization, admixture analysis revealed a number of *argentatus* individuals that were more likely to be characterized with *hyperboreus* genotypes. Admixture analysis indicated that 23.9% of the *argentatus* should be classified as *hyperboreus* and only 2.7% of the *hyperboreus* as *argentatus*, indicating that introgression occurs more frequently from *hyperboreus* to *argentatus*. In agreement with these findings is the fact that the *hyperboreus* which were breeding in a colony in southeast Iceland, which also harboured a large number of *argentatus*, were clustered with all *argentatus* groups. Similarly most of the *argentatus* that were assigned to *hyperboreus* clusters were sampled during time period 1 in southeast Iceland. The observed directional introgression may possibly reflect the different numbers of *argentatus* and *hyperboreus* studied from this area, where hybridization appears to have been common.

The result of this study concurs with numerous reports of introgression and mixed pairing among various gull species in areas of sympatry (e.g. overview in McCarthy 2006). The fact that widespread haplotype sharing between the two species in the European clade was presented more often in areas of sympatry, points to hybridization. If the biphyly was due to shared ancestry, there should be no correspondence to geographic distribution. This biphyly has been detected in other studies (Liebers *et al.* 2004) where introgression was implied along with shared ancestry. As suggested by Crochet *et al.* (2003, 2002), sharing of the most divergent mitochondrial lineages clearly results from introgression and thus the sharing between *hyperboreus* and *argentatus-fuscus* complex was attributed to gene flow. The extensive mitochondrial lineage sharing between *argentatus* and *hyperboreus* observed in this study in Iceland, confirms interspecific gene flow, as claimed by Ingólfsson (1970, 1987, 1993). Where more than one haplotype is found in a species and the less frequent haplotype is identical to the common haplotype in another species, the geographic repartition of haplotypes is indicative of interspecific horizontal transfer. Such events of a specific haplotype obtaining between a recent colonizer (here *argentatus*) and a settled species (here *hyperboreus*) was seen in the North American *marinus* (a recent colonizer), which acquired the *smithsonianus* (the settled one) haplotypes, presumably through hybridization (Crochet *et al.* 2003).

Conclusions

Earlier claims of hybridization between *hyperboreus* and *argentatus* in Iceland (Ingolfsson 1970) based on morphology, were questioned by Snell (1991a, b) who argued that the observed intrapopulation variability in *argentatus* resulted from a founder effect. Snell suggested that the claimed hybrids in Iceland were light-winged *argentatus* originating from Scandinavia and that no hybridization between the two species in Iceland occurs. Along with all the previously mentioned cases of hybridization in gulls, genetic research that has already been published, strongly suggest that gene flow among large white-headed gulls is in fact extensive in many parts of the world (Crochet *et al.* 2002, 2003; Liebers *et al.* 2004, 2006). Thus the haplotype and allele sharing in Icelandic *hyperboreus* and *argentatus* observed in this study should not be surprising. The observed genetic sharing in Iceland followed a geographical pattern and was most obvious in an area where both species were common, most likely as a result of introgression. The genetic analysis in this study based on nuclear and mitochondrial markers support this conclusion, stating that *hyperboreus* and *argentatus* hybridize in Iceland.

Acknowledgements

The authors thank Guðmundur A. Guðmundsson and Gunnar Þór Hallgrímsson, Jóhann Brandsson and Páll Leifsson who provided samples or assisted in the field. We are grateful to our colleagues that provided assistance in the laboratory. We also thank Guðmundur Guðmundsson and his colleagues at the Icelandic Institute of Natural History for assistance with museum samples. This study was supported by the Icelandic Research Council and the Research Grant from the University of Iceland Science Fund.

References

Bandelt HJ, Forster P and Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Barth EK (1968) The circumpolar systematics of *Larus argentatus* and *Larus fuscus* with special reference to the Norwegian populations. *Nytt Mag. Zool* **15**, 1-50.

Barton NH and Hewitt GM (1985) Analysis of Hybrid Zones. *Annual Review of Ecology and Systematics* **16**, 113-148.

Belkhir K, Borsa P, Chikhi L, Raufaste N and Bonhomme F (2004) Genetix version 4.05, Logiciel sous Windows pour la génétique des populations. *Laboratoire Génome, Populations, Interactions. CNRS UMR 5000, Université de Montpellier II, Montpellier, France*.

Bell DA (1996) Genetic differentiation, geographic variation and hybridization in gulls of the *Larus glaucescens-occidentalis* complex. *Condor* **98**, 527-546.

Crochet PA (2000) Genetic structure of avian populations - allozymes revisited. *Molecular Ecology* **9**, 1463-1469.

Crochet PA, Chen JJZ, Pons JM, *et al.* (2003) Genetic differentiation at nuclear and mitochondrial loci among large white-headed gulls: Sex-biased interspecific gene flow? *Evolution* **57**, 2865-2878.

Crochet PA, Lebreton JD and Bonhomme F (2002) Systematics of large white-headed gulls: Patterns of mitochondrial DNA variation in western European taxa. *Auk* **119**, 603-620.

Desjardins P and Morais R (1990) Sequence and Gene Organization of the Chicken Mitochondrial Genome - a Novel Gene Order in Higher Vertebrates. *Journal of Molecular Biology* **212**, 599-634.

Excoffier L and Schneider S (in press) Arlequin ver 3.0: an integrated software package for population genetic data analysis. *Evol Bioinformatics Online*.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes - Application to Human Mitochondrial-DNA Restriction Data. *Genetics* **131**, 479-491.

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. *Distributed by the author. Department of Genetics, University of Washington, Seattle.*

Grant PR and Grant BR (1992) Hybridization of bird species. *Science* **256**, 193-197.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposioum* **41**, 95-98.

Hein J, Schierup MH and Wiuf C (2005) Gene Genealogies, Variation, and Evolution. *Oxford University Press.*

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

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

Hewitt GM (2001) Speciation, hybrid zones and phylogeography - or seeing genes in space and time. *Molecular Ecology* **10**, 537-549.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 183-195.

Ingólfsson A (1970) Hybridization of Glaucous Gulls *Larus hyperboreus* and Herring Gulls *Larus argentatus* in Iceland. *Ibis* **112**, 340-362.

Ingólfsson A (1987) Hybridization of glaucous and herring gulls in Iceland. *Studies in Avian Biology* **10**, 131-140.

Ingólfsson A (1993) The Variably Plumaged Gulls of Iceland. *Auk* **110**, 409-410.

Liebers D, de Knijff P and Helbig AJ (2004) The herring gull complex is not a ring species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 893-901.

Liebers-Helbig D, Knijff P, Sternkopf V and Helbig AJ (2006) Evolution in the Herring Gull species group. *Journal of Ornithology* **147**, 44-44.

Marttinen P, Corander J, Törönen P and Holm L (2006) Bayesian search of functionally divergent protein subgroups and their function specific residues. *Bioinformatics* **22**, 2466.

McCarthy EM (2006) Handbook of Avian Hybrids of the World. *Oxford University Press*.

Nei M (1978) Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. *Genetics* **89**, 583-590.

Newton I (2003) The Speciation and Biogeography of Birds. *Academic press, San Diego*.

Panov EN and Monzikov DG (1999) Intergradation between the herring gull *Larus argentatus* and the southern herring gull *Larus cachinnans* in European Russia. *Zoologichesky Zhurnal* **78**, 334-348.

Randler C (2002) Avian hybridization, mixed pairing and female choice. *Animal Behaviour* **63**, 103-119.

Rhymer JM and Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**, 83-109.

Rogers AR and Harpending H (1992) Population-Growth Makes Waves in the Distribution of Pairwise Genetic-Differences. *Molecular Biology and Evolution* **9**, 552-569.

Slatkin M (1995) A Measure of Population Subdivision Based on Microsatellite Allele Frequencies. *Genetics* **139**, 457-462.

Snell RR (1991a) Interspecific Allozyme Differentiation among North-Atlantic White-Headed Larid Gulls. *Auk* **108**, 319-328.

Snell RR (1991b) Variably Plumaged Icelandic Herring-Gulls Reflect Founders Not Hybrids. *Auk* **108**, 329-341.

Spear LB (1987) Hybridization of Glaucous and Herring-Gulls at the Mackenzie Delta, Canada. *Auk* **104**, 123-125.

Tajima F (1989) Statistical-Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics* **123**, 585-595.

Vallender R, Robertson RJ, Friesen VL and Lovette IJ (2007) Complex hybridization dynamics between golden-winged and blue-winged warblers (*Vermivora chrysoptera* and *Vermivora pinus*) revealed by AFLP, microsatellite, intron and mtDNA markers. *Molecular Ecology* **16**, 2017-2029.

Venables W and Ripley B (1994) Modern applied statistics with S-Plus. *Springer-Verlag, New York.*

Weir BS and Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. *Evolution* **38**, 1358-1370.

Yesou P (1991) The Sympatric Breeding of *Larus* f*uscus, L.* c*achinnans* and *L. argentatus* in Western France. *Ibis* **133**, 256-263.

Chapter II

Analysis of morphological and genetical patterns of herring gull (Larus argentatus) and glaucous gull (Larus hyperboreus) hybridizing in Iceland

Analysis of morphological and genetical patterns of herring gull (*Larus argentatus*) and glaucous gull (*Larus hyperboreus*) hybridizing in Iceland

FREYDÍS VIGFÚSDÓTTIR, SNÆBJÖRN PÁLSSON and AGNAR INGÓLFSSON
Department of Biology, University of Iceland, Sturlugata 7, 101 Reykjavík, Iceland

Abstract

Recent hybridization has been reported for the herring gull (*Larus argentatus*) and glaucous gull (*Larus hyperboreus*) in Iceland, after the colonization of *argentatus* during 1925. Gulls were sampled from different locations in Iceland from three time periods spanning more than 40 years. Morphological variation of the two species are compared with genetical information based on microsatellite and mtDNA data, in order to analyse the nature of hybridization and extent of introgression among the species. Comparisons of both single traits and multivariate analysis points to hybridization and introgression. Putative hybrid individuals are in some cases intermediate in their morphology, in other cases they present one of the parental type, and can only be identified with genetic markers. The extensive hybridization observerd during the first period in southeast Iceland has not had lasting effects of the populations studied. The study supported the fact that phenotypic intermediacy can be indicative of hybridization, as demonstrated in numerous studies on various gull species, and proved to be more effective when morphometric traits were analyzed together. The results also revealed that genetic analysis described the hybrid scenario with more precision and details.

Keywords: Gulls, hybridization, morphometrics, genetics, introgression, Iceland

Introduction

Secondary contact zones between taxa re-expanding from separate glacial refugia during the Holocene and representing all stages of genetic divergence process have been found in diverse kind of organisms (Hewitt 2000). Incomplete reproductive isolation, with or without gene flow, has been documented between many bird species that are phenotypically distinct to some extent (Grant and Grant 1992). Recently, several examples have been documented among avian species e.g. between greater and lesser spotted eagle (Helbig *et al.* 2005), hierofalcon species (Nittinger *et al.* 2007) and golden-winged and blue-winged warblers (Vallender *et al.* 2007). In area of contact, similar imprinting syndromes of related species can actuate hybridization (Grant and Grant 1997; Harris 1970).

In many cases, gulls represent a zone overlap and hybridization (e.g. overview in McCarthy 2006), i.e. broad zones where two species breed sympatrically, but hybridize regularly, even form hybrid swarms or in other instances at low frequency. Such zones clearly indicate secondary contact after periods of allopatric divergence (Hewitt 2000; Hewitt 2004). Liebers *et al.* (2004) suggest that extant taxa of white-headed gulls in the Northern Hemisphere are a result of divergent and reticulate evolution between two ancestral lineages originally separated in two main refugia, a North Atlantic refugium and a continental Eurasian refugium. Incomplete reproductive isolation between these taxa, evolving more rapidly between some lineages than others, has been suggested as an explanation for the observed widespread hybridization among different gull species in areas of contact (Crochet *et al.* 2002; Crochet *et al.* 2003; Liebers *et al.* 2004).

Hybrids were broadly defined by Harrison (1990) as offsprings between individuals from populations which differed by one or more heritable characters. Similarly introgression has been defined as the transfer of genes between species or genetically distinguishable populations (Rieseberg and Carney 1998). For a successful introgression to occur, a gene or genetic marker has to recombine into a new genetic background before it is eliminated by selection against the alleles which it is initially associated (Barton and Hewitt 1985). Genes or genetic markers which may not be associated to any selected genes are therefore expected to introgress more frequently than fitness related traits or markers. Young hybrid zones are of particular interest as they give a valuable opportunity to follow the initial stages of hybridization, but most zones are ancient (Barton and Hewitt 1985; Goodman *et al.* 1999). If hybridization is rare, most hybrid individuals are result of backcrossing.

A recent secondary contact zone is evident in Iceland among the glaucous gull, *Larus hyperboreus* and the European herring gull, *Larus argentatus*. *Argentatus* colonized Iceland around 1925 where *hyperboreus* was already present. Ingólfsson (1970, 1987) used morphological variation to describe the presumed hybridization among the two species. Developing a Hybrid Index based on melanism pattern on the outermost primaries, Ingólfsson allocated hybrids score to individual gulls, evaluating the extent of hybridization in different locations. Apparently pure *hyperboreus* predominated in western Iceland while apparently pure *argentatus* and hybrids were common in southern and eastern Iceland. The variation among the species was extensive and indicated a widespread hybridization. Studying allozyme and morphological variation, Snell (1991a, b) argued against hybridization, claiming that the variation found in *argentatus* in Iceland simply reflected natural variation within the species. Snell argued that the observed intrapopulation variability within *argentatus* in Iceland resulted from a founder effect, suggesting that the claimed hybrids in Iceland were light-winged *argentatus* originating from Scandinavia (but see rebuttal by Ingólfsson 1993). A study of mtDNA and microsatellite variability has revealed an extensive haplotype and allele sharing in Icelandic *hyperboreus* and *argentatus* (Vigfúsdóttir *et al.* in prep.). The observed genetic sharing followed a geographical pattern and was most obvious in an area where both species (based on hybrid index) were common, supporting the conclusion that *hyperboreus* and *argentatus* hybridize in Iceland.

The aim of this paper is to evaluate the extent of hybridization and introgression at the newly established contact zone in Iceland among *hyperboreus* and *argentatus*, as reflected by morphological traits and genetical markers. The temporal range of the study is forty years.

Material and methods

Sampling

Samples were obtained from various localities in Iceland but mainly in the western part (V) and the eastern part (A) (Figure 1). Gulls were assigned to two groups on the basis of primary pattern, those scoring 0.0 - 1.0 in "hybrid index" (Ingólfsson 1970) being called *hyperboreus*, and those scoring 1.1 - 5.0 were termed *argentatus.* This grouping represented reasonably the division of the two species according to names given when birds were handled and given hybrid score. The location in west Iceland (V) was a predominant *hyperboreus* area while the location in east Iceland (A) was a predominant *argentatus* area. Mixed breeding pairs were observed at most colonies, being especially prominent in southeast Iceland (SA), where both types were common (Ingólfsson 1970). Samples originated from three time periods, 1 - 3 and are listed in Table 1 as; species: period: location. Sampling was carried out in the field 2005 – 2006 (period 3) by shooting or Cannon netting, by attracting gulls first by putting out bait near breeding colonies. Other samples were obtained from specimens at The Museum of Natural History in Reykjavik, collected by Ingólfsson in 1964 – 1973 (period 1) and Snell in 1985 – 1986 (period 2) in breeding colonies.

Figure 1. Main sampling locations of gulls investigated. Letters denote location, listed in Table 1.

Table 1. Number of individuals sampled from each location in three time periods* Letters in sample names present; species: period: location. Location corresponds to figure 1.

Samples	Species	Sampling sites	Latitude	Longitude	n
A1A	*Argentatus*	Hromundarey	64°35´35´´	14°18´58´´	46
–ıı–	–ıı–	Horn	64°14´50´´	14°59´53´´	13
–ıı–	–ıı–	Skrudur	64°54´13´´	13°38´17´´	2
–ıı–	–ıı–	Reykjanes	64°00´55´´	22°40´21´´	3
–ıı–	–ıı–	Hjorleifshofdi	63°25´28´´	18°45´38´´	1
A2A	–ıı–	Skrudur	64°54´13´´	13°38´17´´	37
A3A	–ıı–	Reydarfjordur	64°56´03´´	13°41´36´´	25
A3V	–ıı–	Grundafjordur	64°55´31´´	23°15´40´´	3
–ıı–	–ıı–	Reykjanes	64°00´55´´	22°40´21´´	4
H1V	*Hyperboreus*	Bjarnarhafnarfjall	64°59´07´´	23°01´07´´	45
–ıı–	–ıı–	Bulandshofdi	64°56´39´´	23°28´48´´	5
–ıı–	–ıı–	Reykholahreppur	65°30´21´´	22°17´16´´	3
H2V	–ıı–	Bjarnarhafnarfjall	64°59´07´´	23°01´07´´	26
–ıı–	–ıı–	Skrudur	64°54´13´´	13°38´17´´	1
H3V	–ıı–	Grundafjordur	64°55´31´´	23°15´40´´	65
–ıı–	–ıı–	Reydarfjordur	64°56´03´´	13°41´36´´	1
H1A	–ıı–	Horn	64°14´50´´	14°59´53´´	2
–ıı–	–ıı–	Hromundarey	64°35´35´´	14°18´58´´	6
					288

*Time period 1: 1964-1973 sampled by Ingólfsson,
period 2: 1985-1986 sampled by Snell,
period 3: 2005-2006 sampled by authors.

Genetic data.

Genetic data for individuals representing samples in Table 1, was obtained from Vigfúsdóttir *et al.* (in prep.), consisting of 850 bp sequences of mitochondrial cytochrome *b* and genotypes of five microsatellite loci, originally developed by Crochet *et al.* (2003) for *L.a. smithsonianus.* Laboratory methods (isolation, amplification and sequencing) and the genetic analysis are described in Vigfúsdóttir *et al.* (in prep.). Based on the Bayesian clustering method and the admixture analyzes, implemented in BAPS 4.14 (Marttinen *et al.* 2006) individuals that had been defined as *hyperboreus* or *argentatus* based on hybrid index, but which were more likely ($P < 0.05$) to originate genetically from another group were reassigned to species-groups. The individuals that did not rejoin the same group, were taken here for closer inspection to analyse whether the morphometric characters made them more like *hyperboreus* or *argentatus*, to see if any characters were specially diagnostic for the genetical divergence.

Individuals were assigned to 4 groups, based on the hybrid index and the clustering of the genetic data with BAPS. Group 1 includes individuals classified as *argentatus* both according to genetical data and hybrid index. Group 2 holds individuals with *hyperboreus* genetical characteristics but *argentatus* hybrid index. Group 3 holds individuals classified as *hyperboreus* both according to genetical data and the hybrid index. Finally, group 4 holds individuals with *argentatus* genetical characteristics but *hyperboreus* hybrid index. Individuals which belong to group 2 and 4 show signs of hybridization. Either the genes behind the morphological patterns or a number of the genetical markers, i.e. the microsatellites and the mtDNA, may have introgressed between the two species.

The degree of admixture for each individual was estimated further with the admixture coefficents given by the BAPS analysis. The probability of drawing a genotype from the population of the *argentatus* cluster (q_i) was compared to the hybrid index. Individual with q_i value close to 0.5 may have a mixture of alleles and mtDNA from both species.

Morphometric and plumage analysis

Plumage and morphometrics of newly sampled individuals were measured according to Ingólfsson's (1970) protocoll. Measurements of museum skins had been documented and datasheets were kept with corresponding skins. The information was used as it appeared on field datasheets from Ingólfsson and Snell. Hybrid index had not been scored for gulls collected by Snell, thus scored by authors following Ingólfsson (1970) description. The following measurements were taken from samples collected in 2005-2006: Culmen = culmen length from base of feathers on top of bill, Gonys = bill heigth at the gonial angle, Tarsus = length of tarsus, Wing = wing length in outstreched position, Weight = measured with a standarized pesola, HI = Hybrid index for primaries 10 to 6, Bill depth = measured at the proximal border of the nares, Tail = tail length, Mid-toe = length of middle toe. To evaluate the differences between time periods and the sexes, an analysis of variance was conducted. Pairwise correlation among the variables were estimated with the Pearson correlation coefficient. Linear discriminant analysis was conducted to compare the samples for the multivariate data. Individuals wich had missing values were omitted from the analysis. The principle of the method is to find a linear combination of the variables which maximizes the ratio of between-groups variance to within-groups variance (Quinn and Keough 2002).
The morphology of the putative hybrid individuals from groups 2 and 4 was studied by estimating how different, morphologically, these individuals were from the same gender of

genetical group which they belonged to, as reflected by the genetic markers. For example, for each individual in group 2, the probability to obtain as small or smaller culmen length as in group 1, was estimated. Similarly individuals from group 4 were compared to the distributions of group 3.

A classification of the individuals from the four different genetic groups (1-4) based on the multivariate data was summarized with a linear discriminant analysis (LDA).
The LDA analyses were done both for the measured variables, and also on the residuals from the analysis of the variance. The latter method takes into account any differences between the sexes and possible systematic deviations which may have occurred through time.
The contribution of different variables to the discrimination observed with the LDA analysis were studied by correlating each variable with the first two discriminants.

All statistical methods were performed using the statistical software R (http://wwww.r-project.org).

Results

Classification

Genetic analysis revealed number of individuals that had hybrid score of one species but a genetic composition of the other. Using Baps clustering analysis, these individuals were regrouped and identified (Figure 2).

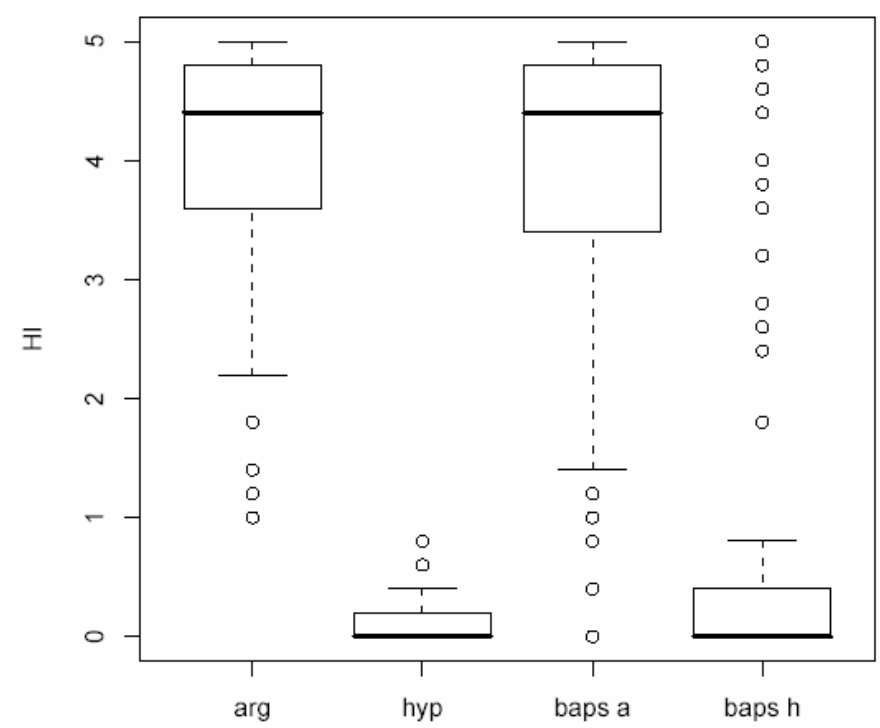

Figure 2. Boxplot of the classification of *argentatus* (arg) and *hyperboreus* (hyp) as a result of hybrid index scoring and as a result of clustering (baps a, baps h) based on the outcome of group level mixture analysis by BAPS (Marttinen *et al.* 2006).

In total there were 32 individuals with *argentatus* hybrid score but had *hyperboreus* genotypes (group 2). The majority (24) of these gulls were sampled in time period 1 in southeast Iceland. Six individuals were sampled in time period 2 in Skrudur (A) and two individuals in time period 3 in Grundafjordur (V). Only 4 individuals had *hyperboreus* hybrid score but *argentatus* genotypes (group 4). They were all from southeast Icleand sampled in time period 1 (H1A).

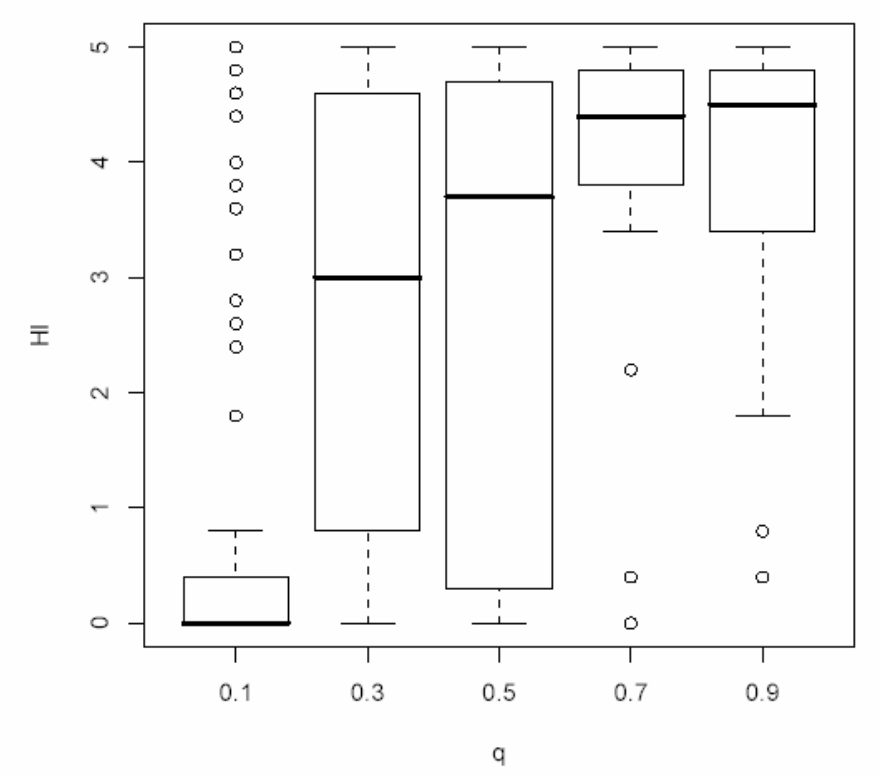

Figure 3. Relationship of admixture coefficents (q_i) per individual and their hybrid index scores. The q_i-value presented are the midvalues. Numbers per each boxplot are and in the same order 164 , 22, 25, 22, and 46 individuals. The proportion of *argentatus*-like and *hyperboreus*-like gulls in the three intermediate boxes are 13.2% and 3.6%, respectively.

The admixture coefficents q_i (here the probability to be assigned to the *argentatus* group) were strongly correlated with the hybrid index scores ($r = 0.648$, $P < 2.2*10^{-16}$) (Figure 3). However several individuals have genotypes not corresponding to their morphology as shown in Figure 2. Admixed genotypes were found more often among *argentatus* (13.2%) than among the *hyperboreus* (3.6%) gulls.

Morphological variation

Morphological measures were overall larger for *hyperboreus* than *argentatus* (Table 2). Among the *argentatus*, the measures were overall larger in birds from the first time period (A1A), as well as the 4 individuals sampled in west Iceland in period 3 (A3V). Scores of hybrid index was lower on average for these groups as well, A1A had 3.87 and A3V had 2.85. *Argentatus* in both these samples originate from areas where *hyperboreus* are equally or more common than *argentatus*. Figure 4, presenting the LDA analyses show this distinction clearly, where A1A and A3V cluster together and separately from A2A and A3A. Tarsus and mid-toe was much bigger in A2A gulls, measured by Snell and were omitted from the LDA

calculations. The average tarsus in Snell's gulls was even larger than the overall average for all the *hyperboreus*. Mid-toe was as well larger for Snell's *argentatus* than the average for *hyperboreus* sampled by Ingólfsson.

Table 2. Morphometric measures of traits for *argentatus* and *hyperboreus* at all time periods. Samples correspond to table 1.

		Larus argentatus					*Larus hyperboreus*				
Trait		**A1A**	**A2A**	**A3A**	**A3V**	***sum:***	**H1V**	**H1A**	**H2V**	**H3V**	***sum:***
Culmen	**n**	**62**	**31**	**24**	**4**	***121***	**51**	**8**	**26**	**52**	***137***
–ıı–	mean	56.67	51.38	50.75	55.03	*53.08*	63.13	59.55	58.69	58.89	*60.43*
–ıı–	S.D.	3.98	2.96	4.20	2.70	*4.55*	3.48	3.56	3.34	4.04	*4.25*
Gonys	**n**	**62**	**31**	**24**	**4**	***121***	**51**	**8**	**26**	**51**	***136***
–ıı–	mean	19.99	18.43	18.86	20.43	*19.38*	20.88	20.39	20.26	20.53	*20.60*
–ıı–	S.D.	1.33	1.23	1.22	1.14	*1.45*	1.30	1.08	1.36	1.25	*1.30*
Tarsus	**n**	**62**	**31**	**24**	**4**	***121***	**52**	**8**	**26**	**52**	***138***
–ıı–	mean	66.83	77.32	62.44	66.35	*68.63*	67.04	70.43	82.97	67.38	*70.41*
–ıı–	S.D.	3.73	4.36	3.92	3.15	*6.63*	3.42	2.12	4.25	3.10	*6.98*
Tail	**n**	**60**	**31**	**-**	**-**	***91***	**7**	**8**	**26**	**-**	***41***
–ıı–	mean	174.63	173.81	-	-	*174.35*	183.29	179.63	186.85	**-**	*184.55*
–ıı–	S.D.	7.33	7.78	-	-	*7.45*	6.68	10.51	8.15	**-**	*8.80*
Wing	**n**	**61**	**31**	**-**	**2**	***94***	**44**	**8**	**26**	**48**	***126***
–ıı–	mean	437.05	417.32	-	433.50	*430.47*	451.28	449.75	443.69	447.69	*448.22*
–ıı–	S.D.	15.34	14.41	-	10.61	*17.50*	13.11	13.23	12.61	11.49	*12.57*
Weight	**n**	**60**	**31**	**24**	**4**	***119***	**52**	**8**	**26**	**52**	***138***
–ıı–	mean	1126.15	999.03	1021.88	1231.25	*1075.54*	1366.06	1266.25	1383.08	1274.04	*1334.68*
–ıı–	S.D.	147.36	119.82	135.20	68.84	*149.79*	227.75	163.70	169.18	167.23	*201.86*
Mid-toe	**n**	**60**	**31**	**-**	**-**	***91***	**4**	**8**	**26**	**-**	***38***
–ıı–	mean	60.13	69.74	-	-	*63.41*	63.50	63.40	75.58	**-**	*71.49*
–ıı–	S.D.	2.94	4.49	-	-	*5.78*	4.73	2.78	3.52	**-**	*6.77*
Bill depth	**n**	**61.00**	**-**	**24.00**	**4.00**	***89.00***	**45**	**8**	**-**	**52**	***105***
–ıı–	mean	18.61	-	16.81	18.68	*18.13*	18.76	19.29	-	18.99	*18.97*
–ıı–	S.D.	1.49	-	1.64	0.91	*1.70*	1.35	0.95	-	1.32	*1.35*
HI	**n**	**61**	**31**	**24**	**4**	***120***	**52**	**8**	**26**	**52**	***138***
–ıı–	mean	3.87	4.62	4.31	2.85	*4.12*	0.17	0.35	0.01	0.05	*0.10*
–ıı–	S.D.	1.08	0.35	0.76	0.53	*0.95*	0.21	0.28	0.04	0.15	*0.19*
Primaries HI	**n**	**63**	**37**	**24**	**8**	***132***	**50**	**8**	**27**	**61**	***146***
10th	mean	3.93	4.74	4.55	3.50	*4.24*	0.47	0.75	0.04	0.14	*0.28*
	S.D.	1.13	0.51	0.61	0.84	*0.98*	0.50	0.46	0.20	0.41	*0.47*
9th	mean	3.78	4.23	4.20	3.50	*3.95*	0.20	0.63	0.00	0.10	*0.15*
	S.D.	1.25	0.76	0.89	1.05	*1.08*	0.41	0.52	0.00	0.30	*0.36*
8th	mean	4.05	4.52	4.20	3.83	*4.19*	0.06	0.25	0.00	0.02	*0.05*
	S.D.	0.97	0.51	0.70	1.17	*0.85*	0.24	0.46	0.00	0.14	*0.21*
7th	mean	4.10	4.81	4.45	3.67	*4.33*	0.02	0.13	0.00	0.00	*0.02*
	S.D.	1.13	0.48	0.83	1.21	*0.99*	0.14	0.35	0.00	0.00	*0.12*
6th	mean	3.44	4.77	4.05	3.21	*3.88*	0.00	0.00	0.00	0.00	*0.00*
	S.D.	1.59	0.76	1.32	1.33	*1.46*	0.00	0.00	0.00	0.00	*0.00*

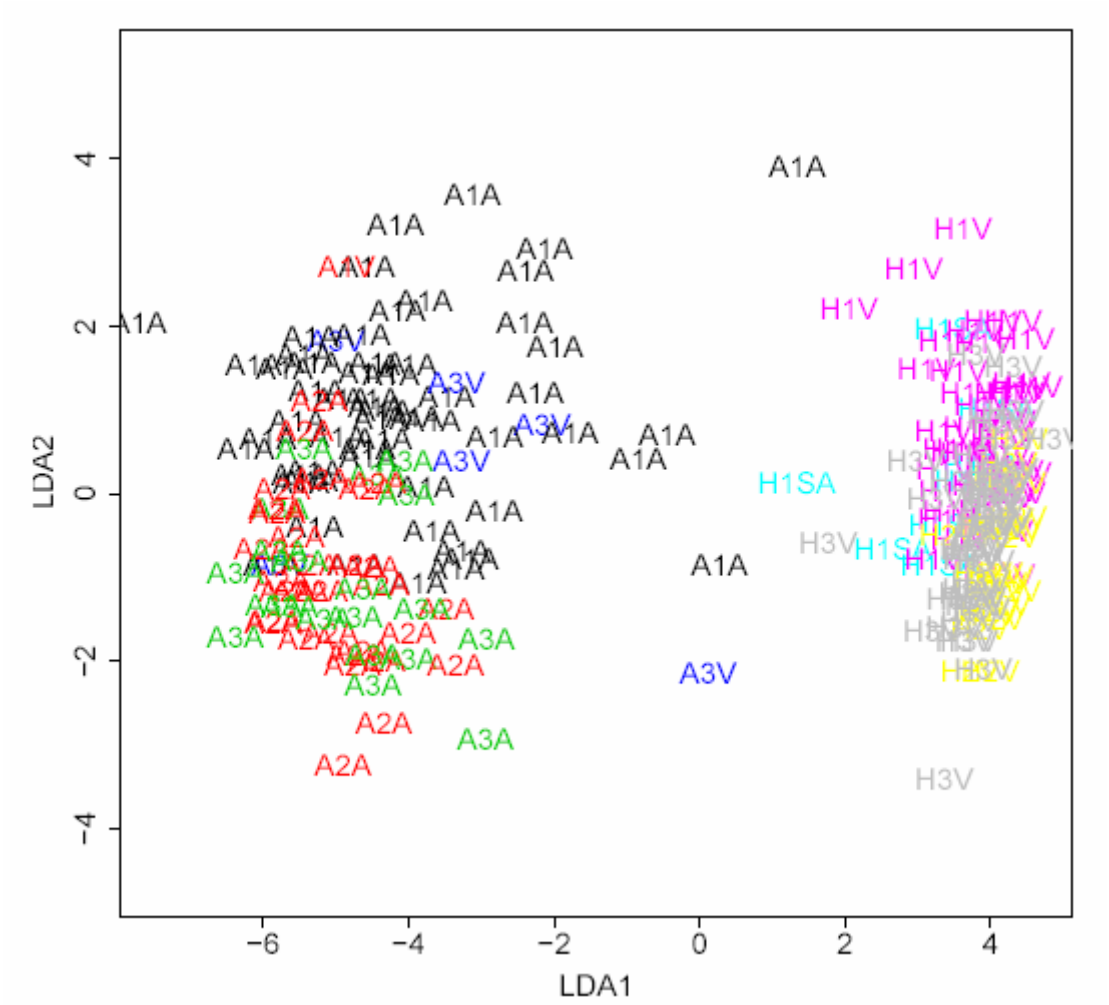

Figure 4. Linear discriminant analysis of the samples based on culmen, gonys, weight and the primary hybrid index scores. Individuals are identified with sample names as presented in Table 1.

Among the *hyperboreus*, the gulls from the first time period (H1A and H1V) showed deviations from other *hyperboreus* (Table 2 and Figure 4*)*. Many of them had small measures of tail and weight and scores of hybrid index was overall higher. This is especially evident for H1A. Tarsus and mid-toe were again much larger for Snell's gulls than the rest.

The apparent morphometric differentation between *argentatus* and *hyperboreus* is obvious and stresses that the classification based on hybrid index scores, which the samples are based on, is quite demonstrative of the grouping. The measurements were negatively correlated to hybrid index scores (r ranging from -0.41 to -0.725, and $P << 0.001$), except for winglength, which was nonsignifcant.

Analysis of variance on the measured variables were conducted on birds which had been sexed, including only birds from the first two time periods. Highly significant differences

between the sexes were found for all variables ($P << 0.001$) in agreement with previous reports (Ingólfsson 1969). Difference between the time periods were also found for all variables ($P << 0.001$) except for tail which had a P value of 0.0134. This is expected as the variables are highly correlated (r ranging from 0.258 to 0.755, and $P < 0.003$) except culmen, gonys, wing and tail lengths.

A further inspection of the 36 misclassified individuals showed how probable it was to obtain such individual trait values from the distribution of the corresponding traits from the species which they had originally been classified with (Figure 5, a). Because of different measurements by Snell, tarsus and mid-toe were based on residuals from the analysis of the variance. The misclassified *argentatus* (32 individuals) showed some signs of deviation from the whole distribution of each trait for all *argentatus* in the study. They had an overall larger measures of culmen, wing and weight. As expected, the median of the hybrid index was in the lower part of the distribution but the range was wide, whereas 34% of these individuals scored more than 4.5 in the index. Other traits were not as distinctive and did not deviate significantly from the whole *argentatus* distribution. Three individuals from group 1 showed peculiar placement in the distribution. They were identified in a linear discriminant analysis (LDA) as described below and are represented with filled dots on Figure 5. These *argentatus*, scoring less than 2.0 in hybrid index, have relatively large measures of all traits compared to other *argentatus*, specially tarsus, wings and mid-toe but a short tail. The 4 misclassified *hyperboreus* did not show as obvious deviations from the mean distribution of each trait for all *hyperboreus* in the study (Figure 5, b). The median of tail and weight was low but the range was wide. The tarsus was the only trait showing any substantial deviation, being at the upper range of the distribution.

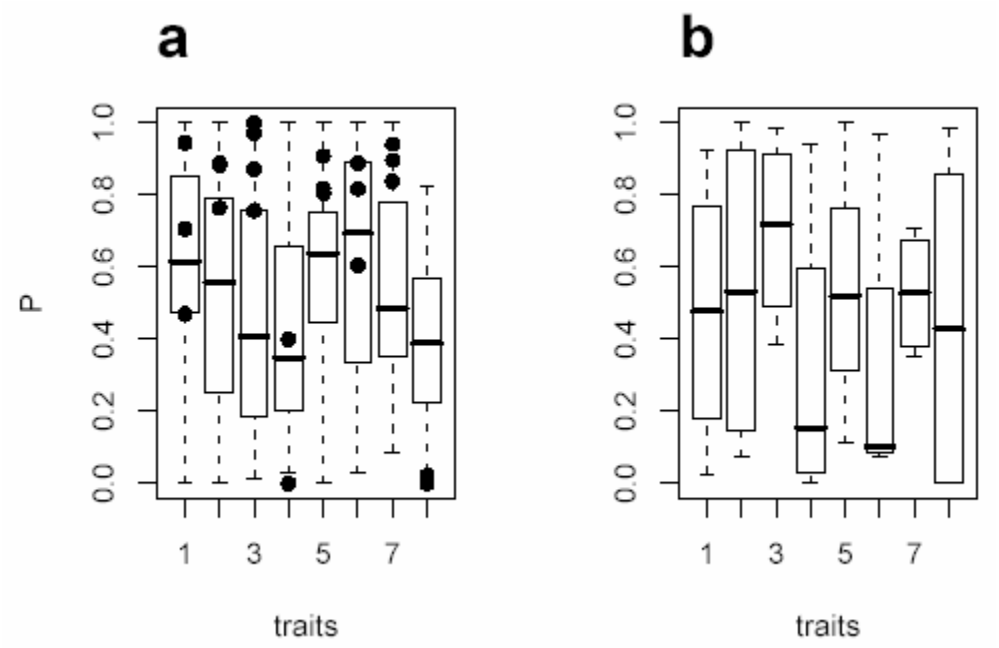

Figure 5. Comparison of putative hybrids with the distribution of putative nonhybrid individuals. *P* presents the probability of obtaining as small or smaller measurement, as observed for the hybrid, within the distribution of the nonhybrid individuals. a) *argentatus*, group 2 compared to group 1. Filled dots gives the probability of individuals from group 1 which clustered in the LDA analysis with group 3 and 4. b) *hyperboreus*, group 4 compared to group 3. The number of the traits is as follows; 1:Culmen, 2:Gonys, 3:Tarsus, 4:Tail, 5:Wing, 6:Weight, 7:Mid-toe, 8:Hybrid index score.

Morphology and genetic differences

Linear discriminant analysis of the four different groups (1- 4), based on the multivariate data, revealed how well the morphology reflected the grouping when maximizing the ratio of between-groups variance to within-group variance. The analysis resulted in two main clusters (Figure 6). The cluster on the left holds individuals with *argentatus* morphology and either with *argentatus* (1) or *hyperboreus* genotypes (2). In the cluster on the right, almost all individuals were genetically and morphologically *hyperboreus* (3), but four individuals had *hyperboreus* genotypes and *argentatus* morphology (4). The individuals in group 4, were somewhat departed along with few from group 3, from the main *hyperboreus* cluster. Between the two main clusters, on the first discriminant axis, few candidates from all groups were found. The first two axis described 85.4% and 8.5% of the between group variance. All measurements, except bill depth were correlated with the first discriminant axis (LDA1), with *r* ranging from 0.237 - 0.502 and *P* ranging from 0.008 - $1.726*10^{-8}$ being highest for mid-toe. Two variables were correlated with the second axis, mid-toe ($r = 0.486$, $P = 2.318*10^{-5}$) and tarsus ($r = 0.486$, $P = 2.35*10^{-8}$). Hybrid index scoring presented strong significantly negative correlation with first axis (LDA1) ($r = -0.484753$, $P = 2.660*10^{-8}$)

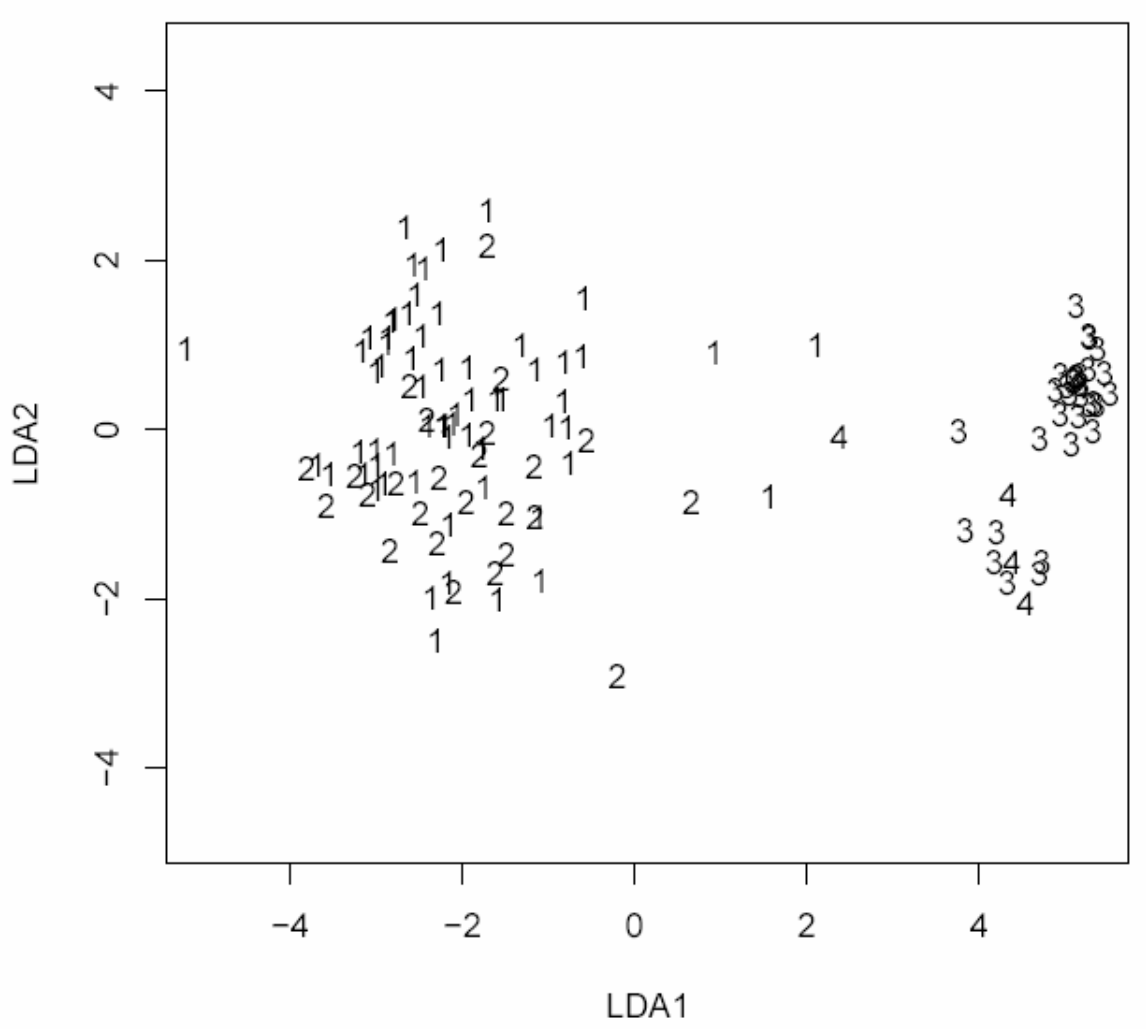

Figure 6. Linear discriminant analysis on the morphology measurements and the hybrid index scores per primay. Groups were defined by genetic and the mean hybrid index scores: (1) *argentatus* with *argentatus* morphology, (2) *argentatus* with *hyperboreus* morphology, (3) *hyperboreus* with *hyperboreus* morphology, and (4) *hyperboreus* with *argentatus* morphology.

When the LDA was just based on the hybrid index information, four *argentatus* individuals of group 1 clustered with the *hyperboreus* individuals. These four individuals had hybrid index scores lower than 2.0 and how they are stationed within the distribution of other measured variables are shown in Figure 5. Only 7.4% of group 2 were correctly classified. All *hyperboreus* individuals from group 3 were correctly classified but all individuals in group 4 clustered with group 3. Thus, the hybrid index was only able to discriminate the putative

hybrid individuals to a very limited extent. When the LDA was based on all characters more hybrid individuals (group 2 and 4) were revealed. 32% of individuals in group 2 and 75% of individuals in group 4 were predicted correctly. A LDA based on the residuals from the analyses of variance gave similar result, however it was not as successful in predicting correctly the putative hybrid individuals, the percentage of correct predictions lowered to 24% and 50% of the cases for groups 2 and 4.

Discussion

Morphological characters clearly distinguish *argentatus* and *hyperboreus* as traditionally defined, although in area of contact like in Iceland where hybridization is evident, the exact boundary between the species is less clear. Hybrids have traditionally been identified by their overall morphological or ecological intermediacy. However, several studies have shown that numbers of intermediate and parental characters expressed in hybrids vary considerable between species, partly reflecting the choice of characters measured (see review by Rieseberg and Carney 1998). This study has revealed that several hybrid individuals present one parental type in morphology, e.g. hybrid score, but genotypic characteristics of the other species. This might be a result of introgression, following generations of hybridizations or backcrossings. Some individuals are clearly intermediate in morphology and might possibly present F1 offsprings from a hybrid crosses.

Some individuals of putative hybrid origin, that were revealed with genetic analysis, had not been clearly distinguished by using only the mean hybrid index scores. Hybrids identified by genetic analyses differed in morphology to some extent from the same morphotype, as observed by the placement in the distribution of measured traits. For *argentatus* putative hybrids, the measures of culmen, wing and weight were indicative of a more *hyperboeus* traits, but for *hyperboreus* putative hybrids the measures of tail and weight were the indicative traits showing a distribution towards a *argentatus* morphometrics. The measures for *hyperboreus* putative hybrids were though just based on 4 individuals, so it might not reflect the situation on a large scale. This indicates that hybrid index was not sufficient in all cases but when other morphometrics were added it gave an indication of the hybridization, giving a more detailed description of the situation.

When all traits were used together in a multivariate analysis, identification of hybrid individuals, which had morphology of one species but the genetic characteristics of the other, were more accurate. Additionally, incidences of individuals with *argentatus* genotypes and hybrid index score lower than 2.0, were presented in the high range of most morphometric distributions for *argentatus* or intermediate phenotypes in the distribution of both species, indicating a more *hyperboreus* morphology. Interestingly groups, showing signs of intermediate morphology, such as A1A and A3V originating from colonies with high numbers of *hyperboreus*, clustered away form A2A, and A3A (both from areas with low

numbers of *hyperboreus*). Similar separation was also found for genetic markers in a previous study (Vigfúsdóttir *et al.* in prep.). Within A1A a large number of individuals were found with *hyperboreus* genotypes, possibly as a result of introgression. However, the whole group was similar in morphology, possibly reflecting segregation of *hyperboreus* genes within the group.

A noteworthy result is that the high instance of introgression or hybridization is not observed among the latter samples from eastern Iceland, indicating that this was an ephemeral event, possibly counteracted by selective forces or recent waves of *argentatus* to Iceland (Vigfúsdóttir *et al.* in prep.). A similar event may be happening in western Iceland if *argentatus* continues to expand their distribution range. A small colony has been found in western Iceland, where both species exist and where pairing appears to be random among them (pers. obs.).

One major problem when studying hybridization is to define the species, as the interbreeding makes the use of the definition of the biological species concept less useful. The traditional classification of *hyperboreus* and *argentatus* has been based on the morphological characters which are clearly separated if one considers e.g. *argentatus* from the British Isles and *hyperboreus* from Iceland (Ingólfsson 1970). However in Iceland there is a continous cline found in the hybrid index despite a bimodal distribution. In this study the boundary was chosen to be 1.0. This selection was arbitrary but the distinction between the two species around the hybrid index score of 1.0 was supported with the BAPS analysis when it was based solely on the hybrid index (data not shown). Additionally, it is difficult to accept that an individual should be a *hyperboreus* with more than 1.0 in hybrid index scoring, as more melanistic markings on primaries giving a higher score, would clearly not resemble a *hyperboreus* according to traditional descriptions of the species would indicate (e.g. Olsen and Larsson 2004; Sibley 2000; Mullarney *et al.* 1999). A hybridization event may result in an increase in hybrid index score above 1, either due to additive effects or especially if pigmentation is a dominant trait, resulting in the classification of a hybrid offspring as *argentatus*. A better understanding of the genetics of the plumage melanism in gulls would help to solve this issue.

Ingólfsson (1970, 1987) identified several morphometric traits and factors that indicated hybridization, additionally to the traits studied here. Additionally, he found *argentatus*-like gulls in southest Iceland, with high hybrid scores or small body size, to have a lower breeding success than other gulls. Ingólfsson found no indication of different adult mortality rate of various hybrid index scores. However, birds with hybrid score between 1.8

and 4.4 were more frequently non-breeders. Ingólfsson also found indications that *hyperboreus* molts its primaries earlier than *argentatus* when living under similar conditions. The significant negative correlation between primary molt score (PMS) and hybrid index found among gulls from southeast Iceland (Horn), was not found in other colonies studied in east Iceland during his years of study, drawing the conclusion that genes for these attributes were not closely linked with those forming primary pigmentation.

A direction in the introgression was observed from *hyperboreus* to *argentatus* in this study. This result differs from the suggestion by Grant and Grant (1997) that such assymetry or unidirectional hybridization occurs when species differ in size, due to preferred matings of females of the smaller species with the males of the larger species. According to that, in this study *hyperboreus* should have attained more mtDNA genotypes from *argentatus,* as female *argentatus* (being smaller) would prefer *hyperboreus* males. A possible explanation can be found in Haldane's rule (Haldane 1922) stating that the heterogamous sex of the hybrids (females in birds) should have decreased fitness. Although the sex-ratios were equal in both hybrid groups (2 and 4), the observed assymetry in the hybridization could possibly reflect that putative female hybrids with *argentatus* mtDNA types experience less survival or fertility, thus not introgressing as freely into *hyperboreus.* Another suggestion, which may apply partly to our data, is that hybridization is generally between the female of the rare species and males of the more common species (Wirtz 1999). This might be valid for the small colony of *hyperboreus* which existed in southeast Iceland during the first time period, however Ingólfsson (1970) did not find any assortative mating with respect to the hybrid index score. Yet another explanation may relate to the species classification and the dominance as discussed above.

In conclustion, this study presents diverse individuals containing a mixture of genes behind the morphological traits and the genetics markers, which otherwise are characterizing the two species. The lower hybrid index scores found in general for Icelandic *argentatus* than found elsewhere in Europe reflects introgression from *hyperboreus* since the arrival of *argentatus* to Iceland around 1925.

Acknowledgements

The authors wish to thank Guðmundur A. Guðmundsson and Gunnar Þór Hallgrímsson, Jóhann Brandsson and Páll Leifsson who provided samples or assisted in the field. We are grateful to our colleagues that provided assistance in the laboratory. We also thank Guðmundur Guðmundsson and his colleagues at the Icelandic Institute of Natural History for assistance with museum samples. This study was supported by the Icelandic Research Council and the Research Grant from the University of Iceland Science Fund.

References

Barton NH and Hewitt GM (1985) Analysis of Hybrid Zones. *Annual Review of Ecology and Systematics* **16**, 113-148.

Crochet PA, Lebreton JD and Bonhomme F (2002) Systematics of large white-headed gulls: Patterns of mitochondrial DNA variation in western European taxa. *Auk* **119**, 603-620.

Crochet PA, Chen JJZ, Pons JM, Lebreton J-D, Herbert PDN and Bonhomme F (2003) Genetic differentiation at nuclear and mitochondrial loci among large white-headed gulls: Sex-biased interspecific gene flow? *Evolution* **57**, 2865-2878.

Goodman SJ, Barton NH, Swanson G, Abernethy K and Pemberton JM (1999) Introgression through rare hybridization: A genetic study of a hybrid zone between red and sika deer (genus *Cervus*) in Argyll, Scotland. *Genetics* **152**, 355-371.

Grant PR and Grant BR (1992) Hybridization of bird species. Science 256, 193-197.

Grant PR and Grant BR (1997) Hybridization, sexual imprinting, and mate choice. *American Naturalist* **149**, 1-28.

Haldane JBS (1922) Sex-ratio and unisexual sterility in hybrid animals. **J. Genetics 12**, 101-109.

Harris M (1970) Abnormal migration and hybridization of *Larus argentatus* and *L. fuscus* after interspecies fostering experiments. *Ibis* **112**, 488-498.

Harrison R (1990) Hybrid zones: windows on evolutionary process. *Oxford Surveys in Evolutionary Biology* **7**, 69–128.

Helbig AJ, Seibold I, Kocum A, Liebers D, Irwin J, Bergmanis U, Meyburg BU, Scheller W, Stubbe M and Bensch S (2005) Genetic differentiation and hybridization between greater and lesser spotted eagles (Accipitriformes: *Aquila clanga, A-pomarina*). *Journal of Ornithology* **146**, 226-234.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907-913.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 183-195.

Ingólfsson A (1969) Sexual dimorphism of large gulls (*Larus* spp.). *Auk* **86**, 732-737.

Ingólfsson A (1970) Hybridization of Glaucous Gulls *Larus hyperboreus* and Herring Gulls *Larus argentatus* in Iceland. *Ibis* **112**, 340-362.

Ingólfsson A (1987) Hybridization of glaucous and herring gulls in Iceland. *Studies in Avian Biology* **10**, 131-140.

Ingólfsson A (1993) The Variably Plumaged Gulls of Iceland. *Auk* **110**, 409-410.

Liebers D, de Knijff P and Helbig AJ (2004) The herring gull complex is not a ring species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 893-901.

Marttinen P, Corander J, Törönen P and Holm L (2006) Bayesian search of functionally divergent protein subgroups and their function specific residues. *Bioinformatics* **22**, 2466.

McCarthy EM (2006) *Handbook of Avian Hybrids of the World*. Oxford University Press.

Mullarney K, Svensson L, Zetterström D and Grant P (1999) *The Collins bird guide* HarperCollins, London.

Nittinger F, Gamauf A, Pinsker W, Wink M and Haring E (2007) Phylogeography and population structure of the saker falcon (*Falco cherrug*) and the influence of hybridization: mitochondrial and microsatellite data. *Molecular Ecology* **16**, 1497-1517.

Olsen K and Larsson H (2004) *Gulls of Europe, Asia and North America,* 2nd edn. Princeton University Press, Princeton, New Jersey.

Quinn GP and Keough MJ (2002) *Experimental design and data analysis for biologists* Cambridge University Press.

Rieseberg LH and Carney SE (1998) Tansley Review No. 102 Plant hybridization. *New Phytologist* **140**, 599-624.

Sibley D (2000) *The Sibley guide to birds* Alfred A. Knopf, Inc., New York.

Snell RR (1991a) Interspecific Allozyme Differentiation among North-Atlantic White-Headed Larid Gulls. *Auk* **108**, 319-328.

Snell RR (1991b) Variably Plumaged Icelandic Herring-Gulls Reflect Founders Not Hybrids. *Auk* **108**, 329-341.

Vallender R, Robertson RJ, Friesen VL and Lovette IJ (2007) Complex hybridization dynamics between golden-winged and blue-winged warblers (*Vermivora chrysoptera* and *Vermivora pinus*) revealed by AFLP, microsatellite, intron and mtDNA markers. *Molecular Ecology* **16**, 2017-2029.

Wirtz P (1999) Mother species-father species: unidirectional hybridization in animals with female choice. *Animal Behaviour* **58**, 1-12.

Acknowlegdements

Special thanks go to my supervisors, Associate Professor Snæbjörn Pálsson and Professor Agnar Ingólfsson, for all their guidance in all matters concerning this work, both theoretical and practical. I am grateful to numerous colleagues that provided assistance in the laboratory and specially to Professon Einar Árnason for providing access to various laboratory material and equipment. I would like to thank Dr. Guðmundur A. Guðmundsson at the Icelandic Institute of Natural History in Reykjavík and Gunnar Þór Hallgrímsson a Ph.D. student at the University of Iceland for their altruistic contribution and assistance with Cannon netting and measuring gulls. I also thank Jóhann Brandsson in Kulusuk, Greenland and Páll Leifsson in Eskifjordur, East Iceland, who also provided samples. I also thank Dr. Guðmundur Guðmundsson and his colleagues at the Icelandic Institute of Natural History for assistance with museum samples as well as for his help with database handling. This study was supported by the Icelandic Research Council and the Research Grant from the University of Iceland Science Fund.