The Herring Gull Complex (*Larus argentatus - fuscus - cachinnans*) as a Model Group for Recent Holarctic Vertebrate Radiations

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Abstract Under what circumstances speciation in sexually reproducing animals can occur without geographical disjunction is still controversial. According to the ring species model, a reproductive barrier may arise through "isolation-by-distance" when peripheral populations of a species meet after expanding around some uninhabitable barrier. The classical example for this kind of speciation is the herring gull (*Larus argentatus*) complex with a circumpolar distribution in the northern hemisphere. An analysis of mitochondrial DNA variation among 21 gull taxa indicated that members of this complex differentiated largely in allopatry following multiple vicariance and long-distance colonization events, not primarily through "isolation-by-distance".

In a recent approach, we applied nuclear intron sequences and AFLP markers to be compared with the mitochondrial phylogeography. These markers served to reconstruct the overall phylogeny of the genus *Larus* and to test for the apparent biphyletic origin of two species (*argentatus, hyperboreus*) as well as the unexpected position of *L. marinus* within this complex. All three taxa are members of the herring gull radiation but experienced, to a different degree, extensive mitochondrial introgression through hybridization. The discrepancies between the mitochondrial gene tree and the taxon phylogeny based on nuclear markers are illustrated.

1 Introduction

Ernst Mayr (1942), based on earlier ideas of Stegmann (1934) and Geyr (1938), proposed that reproductive isolation may evolve in a single species through

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"isolation-by-distance" when peripheral populations meet after expanding around a large, uninhabitable area. This mode of speciation through "circular overlap" (Mayr 1942) was later termed the "ring species" model (Cain 1954). Geographic overlap between taxa that are elsewhere connected through interbreeding populations is an essential element of this model, because it is ongoing gene flow that distinguishes ring species from cases of allopatric speciation that happen to be arranged in a roughly circular fashion (Irwin et al. 2001a).

The classical example of the ring species model was originally based upon the herring gull complex (Mayr 1942). This group comprises more than 20 taxa of large gulls (Haffer 1982) which together occupy a circumpolar breeding range in the northern hemisphere. Phenotypically, taxa differ most obviously in body size and in

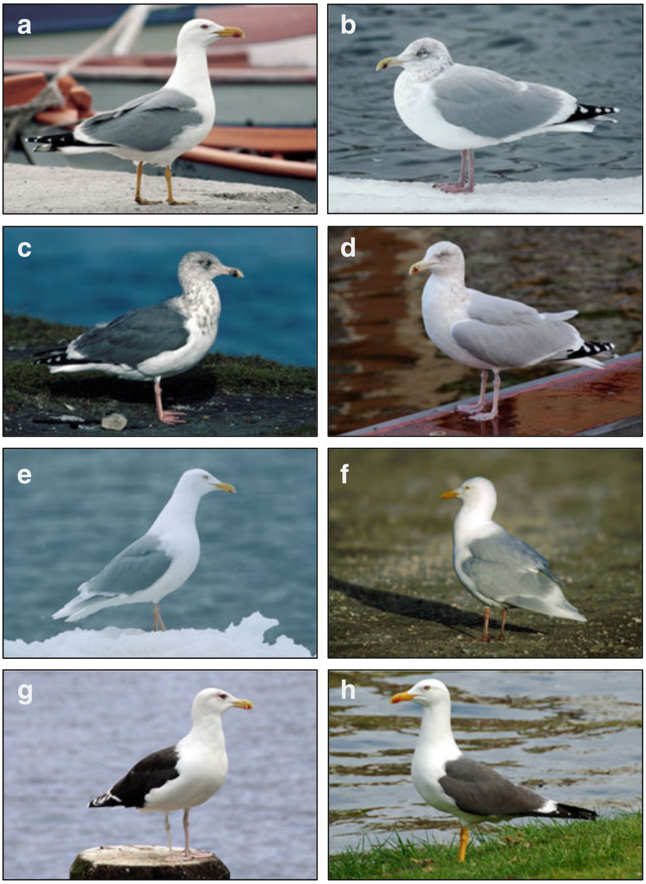

Fig. 1 Examples of phenotypic variation within the herring gull complex. (**a**) *L. michahellis*, Lesbos, Greece, (**b**) *L. smithsonianus*, Newfoundland, Canada, (**c**) *L. a. argentatus*, Rotterdam, Netherlands, (**d**) *L. a. argenteus*, Leiden, Netherlands, (**e**) *L. hyperboreus,* Svalbard, (**f**) *L. hyperboreus*, Churchill, Canada, (**g**) *L. marinus,* Greifswald, Germany, (**h**) *L. fuscus,* Leiden, Netherlands. Photos: P. de K. (**a**–**c**, **e**, **f**), V.S. (**d**, **g**, **h**)

the darkness of their dorsal plumage (see Fig. 1), which varies from pale gray to black (Malling Olsen and Larsson 2003). According to Mayr's (1942) model, the group originated in the Aralo-Caspian region, from where gulls spread in three directions (Fig. 2): (1) west via the Mediterranean into the Atlantic giving rise to Mediterranean (*michahellis*) and Atlantic (*atlantis*) herring gulls; (2) east toward Inner Asia giving rise to the Mongolian gull (*mongolicus*) and (3) north to the Arctic Ocean. Along the north Eurasian coasts, the ancestral population expanded both ways: (1) west across Scandinavia towards Britain and Iceland differentiating into dark-mantled lesser black-backed gulls (*fuscus*), and (2) east all the way to the North Pacific giving rise to progressively paler-mantled forms, *taimyrensis* (Taimyr), *birulai* and *vegae* (eastern Siberia), and into North America (*smithsonianus*). Following the last Glacial Maximum, North American herring gulls are supposed to have crossed the North Atlantic and invaded Europe, where

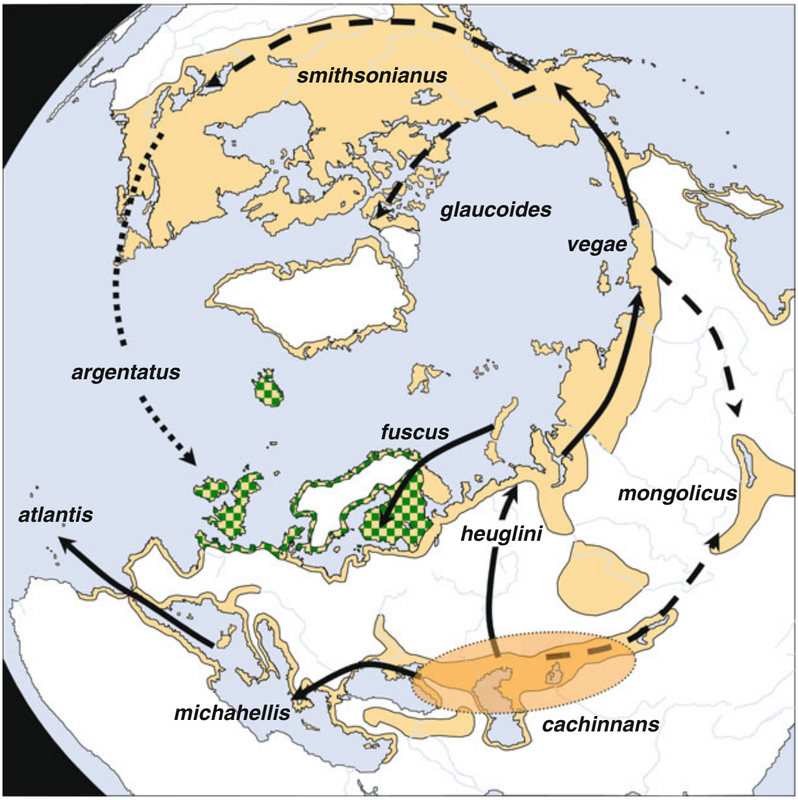

Fig. 2 Mayr's (1942) ring species model about the differentiation and colonization history of the herring gull complex. Mayr assumed a single Aralo-Caspian refugium (*pale orange oval*) and a most recent invasion of herring gulls from North America to Europe, where they now overlap with lesser black-backed gulls (*checker-board pattern*). *Arrows* indicate inferred colonization routes with temporal progression from ancient to most recent events indicated by *solid*, *broken* and *stippled arrows*, respectively

they now overlap with lesser black-backed gulls (Mayr 1942; Geyr 1938). Mayr envisioned all taxa of the circumpolar chain to be connected by gene flow, while herring and lesser black-backed gulls in Europe, the hypothetical endpoints of the ring, have reached full reproductive isolation and now coexist as distinct species.

Previous attempts to test the ring species model in these gulls were inconclusive due to the low amount of variation recovered from allozymes (Snell 1991) and short, conservative segments of mitochondrial DNA (Crochet et al. 2002). Our work has shown for the “yellow-legged” *L. cachinnans* subgroup (Liebers et al. 2001) and for the dark-mantled *L. fuscus* subgroup (Liebers and Helbig 2002) that partial reproductive barriers have arisen in situ between neighboring taxa both within the northern (Arctic Ocean – NE Atlantic) and the southern (Mediterranean – Aralo-Caspian) chain of taxa that Mayr (1942) hypothesized to be interconnected by gene flow.

There are a few well-documented cases of ring species (see, e.g., Irwin et al. 2001b for another avian example), most of which indicate that repeated allopatric fragmentation of large, roughly circular ranges is likely to lead to the evolution of multiple reproductive barriers *before* circular overlap and isolation-by-distance do. In particular, the well-known *Ensatina* complex of salamanders in western North America (Wake 1997), as well as our results obtained so far for the herring gull complex, best fit such a scenario. This means that fragmentation of circular ranges, most likely due to repeated episodes of glacial advance and retreat, may be an important mechanism in the early stages of a radiation in diverse groups of vertebrates.

We have already completed a comprehensive study of mitochondrial DNA (mtDNA) variation of the herring gull complex (Liebers et al. 2004). The aim of the present project, funded by the German Research Foundation for 4 years, was to use nuclear genetic markers (AFLP genotyping, intron sequences) to test a number of hypotheses derived from the previous study. The AFLP technique has been shown to be also effective in a number of different groups of birds, e.g., in gulls (de Knijff et al. 2001), *Phylloscopus* warblers (Bensch et al. 2002), house finch (Wang et al. 2003), *Hippolais* warblers (Secondi et al. 2006), New World crossbills (Parchman et al. 2006), and golden- and blue-winged warblers (Vallender et al. 2007). The same holds for the resolution of autosomal (intron) sequences, not only for closely related avian species groups such as *Phylloscopus* warblers (Bensch et al. 2006) and *Aquila* eagles (Helbig et al. 2005), but also for resolving higher order Avian phylogenies (Hackett et al. 2008).

2 State of the Art

We investigated the phylogeography of 20 northern hemisphere gull taxa (Fig. 3) and one southern large gull (*L. dominicanus*) using 1.5 kb of mitochondrial DNA sequence. This included the entire cytochrome b gene and a hypervariable part of the mitochondrial control region (HVR-1), which had previously been shown to be highly informative about recent evolutionary differentiation of various taxa (Liebers and Helbig 1999, 2002; Liebers et al. 2001).

The haplotype network (Fig. 4) shows extensive genetic divergence within this complex of gulls. Analysis of molecular variance (Excoffier et al. 1992) indicated strong segregation of haplotypes along taxonomic, i.e., phenotypically defined, boundaries. The significant taxonomic and geographic structure enables us to interpret the evolutionary history of these gulls based on mitochondrial genetic variation. The western gull (*Larus occidentalis*) of the North American west coast (range not shown in Fig. 3) was found to have highly divergent haplotypes relative to all other taxa in this study and is regarded as the outgroup. Nested Clade Analysis (NCA; Templeton 1998) indicated that the ancestral ingroup population was divided by an allopatric fragmentation event leading to the evolution of two major clades: clade I centered in the North Atlantic and clade II with a circumpolar distribution. Assuming a cytochrome b divergence rate of 1.6% per million years (Fleischer et al. 1998), the initial vicariance event occurred some 308,000 (95% CI: 102,000–602,000) years ago. *L. argentatus* and *L. cachinnans* are the two taxa currently containing the most highly divergent, and the earliest-branching, haplotype lineages (Fig. 4). This indicates that they are direct descendants of the two ancestral populations. If current breeding ranges are any indication, ancestors of clade I probably lived in the north-eastern Atlantic (current range of *argentatus*), those ancestral to clade II lived in the Aralo-Caspian region (current range of *cachinnans*; Fig. 5).

As indicated by NCA, the Aralo-Caspian population (ancestors of clade II) spread by contiguous range expansion toward the north Eurasian coast, then west up to Britain and Iceland (*fuscus* range) and east throughout northern Siberia (*vegae, schistisagus*) and North America (*smithsonianus, glaucescens, glaucoides;* Fig. 5). In accordance with Mayr's theory, sharing of haplotypes between adjacent taxa in this circumpolar range indicates ongoing gene flow. However, we find no support for the key element of the ring species hypothesis, i.e., a transatlantic invasion of North American herring gulls *(smithsonianus)* into Europe. No haplotypes typical of, or derived from, Nearctic *smithsonianus* were found anywhere in the European *argentatus* population, not even in Iceland. The endpoints of the circumpolar ring of interbreeding taxa, therefore, do not overlap. Furthermore, yellow-legged gulls of the Atlantic Islands (*atlantis*), Mediterranean Sea (*michahellis*) and Asia Minor (*armenicus*) are derived from North Atlantic (clade I), not Aralo-Caspian ancestors (contra Mayr 1942).

Two more aspects of the mitochondrial phylogeny are surprising. First, three distinct species previously thought to derive from phylogenetically older ancestors are nested within the herring gull complex: great black-backed gull (*L. marinus*) and glaucous gull (*L. hyperboreus*), which overlap extensively in breeding range between themselves and with other members of the complex, and the southern hemisphere kelp gull (*L. dominicanus*). The latter evolved via long-distance colonization from the same ancestral population as the lesser black-backed gull (Fig. 4), suggesting that the ancestors of kelp gulls were highly migratory as nominate lesser black-backed gulls still are today.

Fig. 3 Breeding ranges and sampling locations (*dots*) of the gull taxa investigated. For reasons of clarity, extensively overlapping ranges are shown on different maps, e.g., for *L. argentatus* in Fig. 8 and for *L. marinus*, and *L. hyperboreus* in Fig. 9. Ranges of *L. occidentalis* and *L. dominicanus* are not shown

Second, two taxa are biphyletic in the mtDNA network: populations of European *argentatus* (pale blue in Fig. 4) and of *hyperboreus* (dark gray) each contain haplotypes of both major clades. This can be explained by retention of ancestral polymorphisms and/or by mitochondrial gene flow that occurred after the initial split into two separate refugia.

In the case of *argentatus*, there is evidence that both processes were involved. Nuclear AFLP markers (de Knijff et al. 2001) showed *argentatus* to be most closely related to clade I taxa (*atlantis*/*michahellis*). Within clade I, *argentatus* haplotypes within the *marinus*-cluster probably reflect ancient polymorphism. The geographically widespread occurrence of clade II haplotypes in extant *argentatus* populations appears to be the footprint of a past gene flow episode. Birds derived from the Aralo-Caspian refugium, possibly members of a pre-*heuglini* population, must have hybridized, perhaps briefly, with the ancestral *argentatus* population from the North Atlantic. The introgressed clade II mitochondrial lineage persisted and diversified to this day in the *argentatus* population.

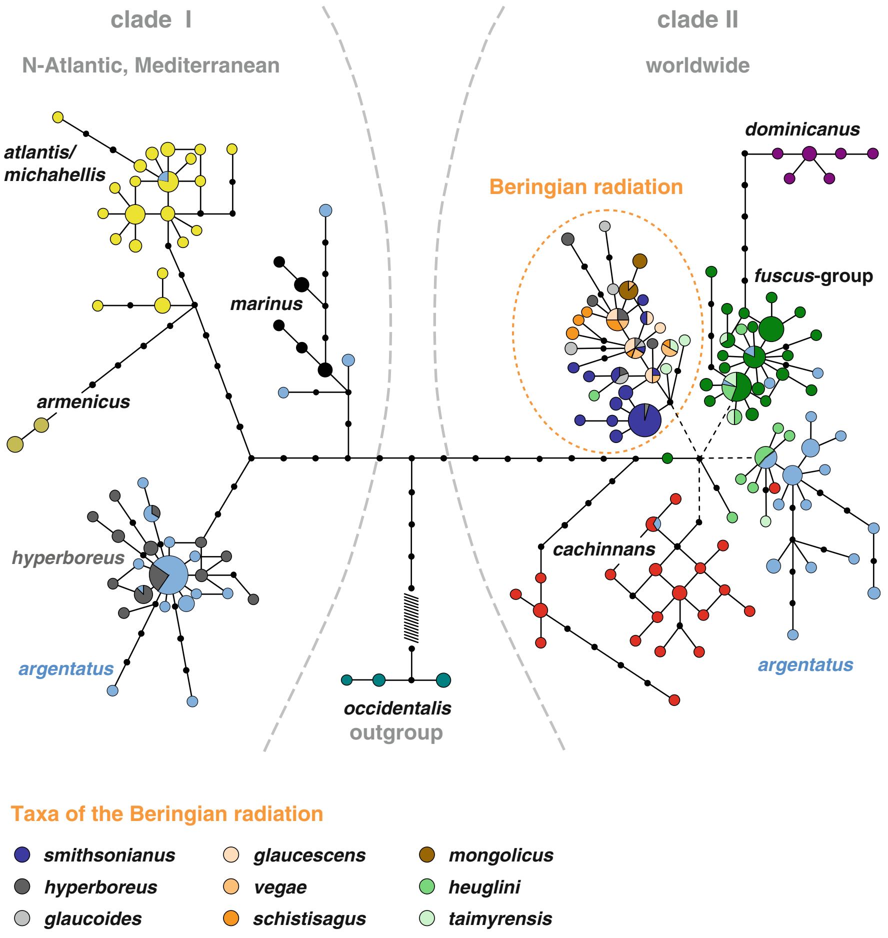

Fig. 4 Median-joining network (Bandelt et al. 1999) of 160 concatenated mtDNA haplotypes (cytochrome b, HVR-1) identified in this study. *Larus occidentalis* was designated as the outgroup. *Colors* represent taxa as shown in Fig. 3, Fig. 8 (*L. argentatus*), and Fig. 9 (*L. marinus* and *L. hyperboreus*)

The apparent biphyly of glaucous gulls (*hyperboreus*) is more problematic. In Palearctic birds, we found only haplotypes closely related to or shared with European *argentatus* (clade I), while Nearctic *hyperboreus* only contained a variety of haplotypes shared with North American and Pacific taxa (clade II). Hybridization between glaucous and herring gulls has been observed in Iceland (Ingolfsson 1970; Vigfúsdottir et al. 2008) and north-western Canada (Spear 1987) and may have led to mitochondrial introgression.

We found no close relationship between mitochondrial genetic distance and reproductive isolation. The most divergent taxon in terms mitochondrial DNA, *L. occidentalis*, hybridizes extensively with one of the ingroup taxa, *L. glaucescens*,

Fig. 5 Alternative model of the colonization history of the herring gull complex based on the mitochondrial DNA-sequences. Two ancient refugia are inferred (*pale green* and *pale orange oval*). Current ranges of taxa derived from Atlantic refugium are shown in *green*, those derived from Aralo-Caspian refugium are in *orange*, *checker-board pattern* shows areas of overlap. No invasion of herring gulls from North America to Europe occurred. *L. marinus* developed reproductive isolation in allopatry (probably in north-eastern North America) before making secondary contact with North American *smithsonianus* and Eurasian *argentatus*/*fuscus*. Two separate colonization events from the Atlantic into the Mediterranean led to the differentiation of *armenicus* and, much later, *michahellis*. *Arrows* as in Fig. 2

along the west coast of North America (Bell 1997). On the other hand, our data do not support the traditional view of great black-backed gull (*L. marinus*) being an outgroup relative to the herring gull complex. Although *L. marinus* is clearly reproductively isolated from all species it co-occurs with, in the mitochondrial network it is nested among taxa several of which hybridize: *argentatus* x *hyperboreus* (Ingolfsson 1970; Spear 1987), *michahellis* x *graellsii* (van Swelm 1998), *cachinnans* x *argentatus* (Panov and Monzikov 1999), and, earlier in the twentieth century, *argentatus* x *fuscus* (Tinbergen 1953).

3 Recent Progress

Mitochondrial DNA alone, due its maternal and clonal mode of inheritance, potentially being subject to selective sweeps and stochastic effects of lineage sorting, is not sufficient to reconstruct robustly the phylogeography and sequence of divergence events leading to a recent radiation such as that of the herring gull complex (e.g., Sattler and Braun 2000; Ballard and Whitlock 2004). We therefore studied and still work on autosomal, i.e., biparentally inherited nuclear markers, in order to

- Compare the nuclear genomic with the mitochondrial phylogeny.
- Compare levels of nuclear versus mitochondrial gene flow.
- Identify classes of markers that segregate at the earliest stages of the speciation process and may thus be generally applicable to the study of recent radiations in birds and perhaps other vertebrates.

3.1 Phylogenetic Framework for the Herring Gull Complex

Question: What species not included in our study so far might be part of the herring gull complex? The herring gull complex has traditionally been seen as a northern circumpolar group, but we have already shown that one southern hemisphere species (*L. dominicanus*) is in fact part of it. This may well be true for a number of other species (Fig. 6) that so far were not regarded as part of the complex either because of their smaller body size (*L. californicus, L. canus*) or due to their southern hemisphere distribution (*L. pacificus*).

To answer this question and, equally importantly, in order to check for congruence between phylogenetic signal between mitochondrial and nuclear genomes, we broadened the taxon sampling both for mitochondrial sequences (cytochrome b gene, HVR-1) and nuclear intron sequences (LDH intron 3, VLD intron 9, GAP intron 11, BRM intron 15) to provide a detailed phylogenetic framework for the herring gull complex (Sternkopf et al., in preparation).

Results: It turned out that *L. californicus*, although clearly smaller than all other members of the herring gull group identified so far, is indeed a member of this group (Fig. 7), while *L. canus* and *L. delawarensis* together form the sister group of the complex. More specifically*, L. californicus* is a member of clade II of the overall mitochondrial network (see Fig. 4) and is derived within the Beringian radiation which diversified primarily in the North Pacific–North American region (de Knijff et al. 2005; Sternkopf et al., in preparation).

The large Australian species *L. pacificus*, although superficially similar to *L. occidentalis* and *L. marinus* of the Holarctic and *L. dominicanus* of the southern hemisphere, is not a member of the complex. It forms a clade with *L. crassirostris* and *L. belcheri*, two other Pacific species with which it shares a black subterminal tail band in adult plumage. This character does not occur in adult plumages in the herring gull complex.

Fig. 6 Known members and new candidates for the herring gull radiation. (**a**) The kelp gull (*L. dominicanus*) is a southern hemisphere representative of the herring gull complex, nested within clade II. (**b**) The Pacific gull (*L. pacificus*) resembles the kelp gull at first glance. It inhabits the coasts of Australia and Tasmania. (**c**) Common gull (*L. canus*) and (**d**) California gull (*L. californicus*) are smaller gulls that breed throughout the Palearctic and in interior North America, respectively. Photos: (**a**) E. Stich, King George Island, Antarctica. (**b**) N. Murray, Phillip Island, Australia. (**c**) P. de K., Rotterdam, Netherlands. (**d**) P. de K., Palo Alto, California

Overall, the phylogeny of the Laridae has been much better resolved than in previous attempts (Crochet et al. 2000; Pons et al. 2005). Monophyly of the genus *Larus* was fully supported (blue triangle in Fig. 7), but only to the exclusion of the little gull (previously called *L. minutus* but renamed as *Hydrocoleus minutus*; cf. Helbig 2005). This species is a member of the basal clade of non-*Larus* gulls (*Rissa, Xema, Pagophila, Creagrus*). Our results strongly support a sister taxon relationship between little gull and Ross's gull (*Rhodostethia rosea)*. As a taxonomic consequence, we propose to exclude little gull from the genus *Larus* and instead group it together with the Ross's gull into the genus *Hydrocoleus* (Sternkopf et al., in preparation).

Within the mitochondrial control region, a larger insert (31 base pairs) was shared by the "masked gulls" (*L. genei–ridibundus* group), yielding additional support (beyond single nucleotide variation) for the monophyly of this group. The biphyly of large gulls is also apparent in the combined mitochondrial and nuclear genomic sequences, further supporting the two-refugia model of their phylogenetic history (Fig. 5).

3.2 Population History of "polyphyletic" Taxa in the Mitochondrial Gene Tree

Question: Several taxa were represented by a biphyletic pattern in the mitochondrial–genetic network, most notably *L. argentatus* and *L. hyperboreus* (see Fig. 4), but as

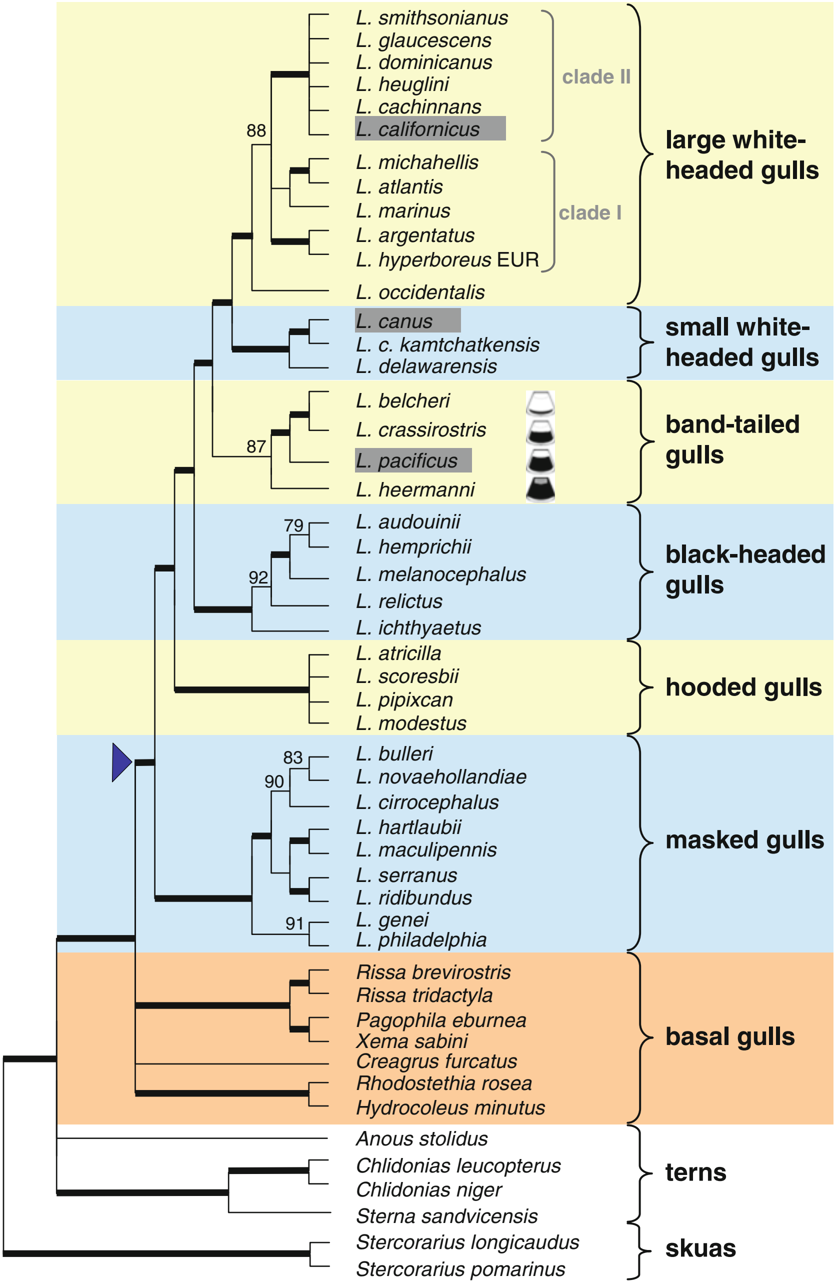

Fig. 7 Bayesian consensus tree of gulls of the family Laridae based on concatenated mitochondrial (cytochrome b, HVR-1) and nuclear intron (LDH 3, VLD 9, GAP 11, BRM 15) sequences. Numbers on branches are support of Bayesian posterior probabilities (GTR+γ model, 20,000 generations), *thick* branches indicate support values higher than 95. *L. californicus, L. canus,* and *L. pacificus*, three species suspected as possible members of the herring gull complex, are *shaded*. Monophyly of the genus *Larus* is shown by a *blue triangle*

we discovered in the meantime, this is now also true of *L. marinus* (Sternkopf et al., in preparation). The question arises whether individuals belonging to different clades in the mitochondrial gene tree but co-occurring in the same geographic area form a single or separate reproductive communities?

In the mitochondrial network, *L. argentatus* (pale blue in Fig. 4) shows a biphyletic origin and strong geographic substructure within its Palearctic range. Clade I haplotypes (green portion in Fig. 8) predominate in the northern populations, clade II haplotypes in the south (orange portion in Fig. 8; Sternkopf et al., in preparation).

Glaucous gull *L. hyperboreus*, which is also biphyletic in the network (dark gray in Fig. 4), shows an even more pronounced segregation of mitochondrial haplotypes (Fig. 9). All North American birds exhibit clade II haplotypes (orange portion in Fig. 9), while European populations, except two (wintering, not breeding) birds

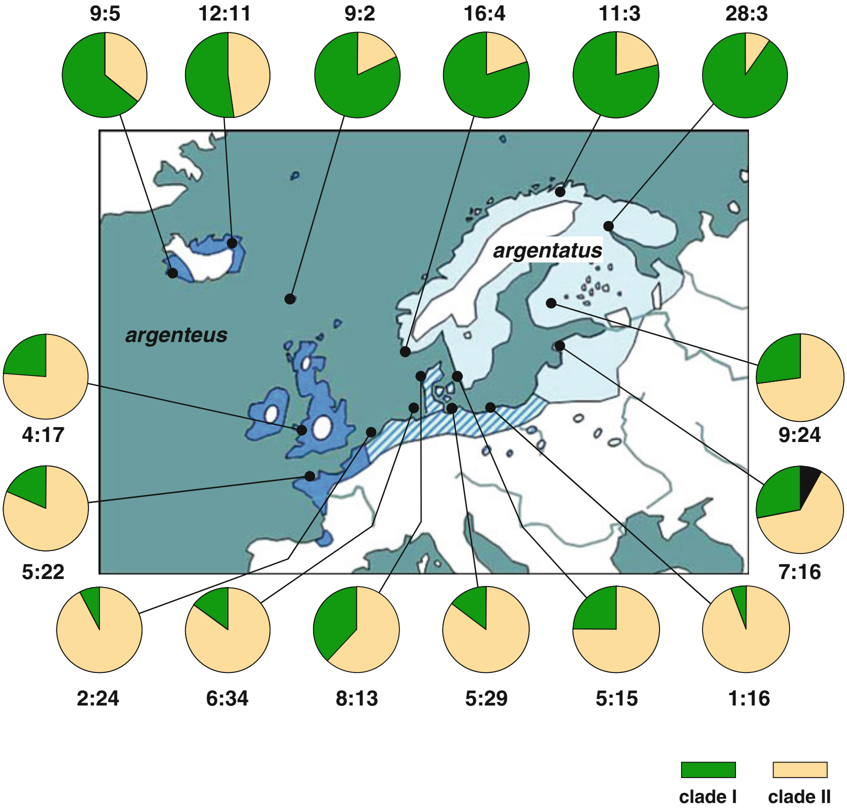

Fig. 8 Breeding ranges, sampling locations (*dots*) and haplotype composition (*pie charts*) of herring gulls throughout Europe ($n = 377$). Areas of intermixing between the two subspecies *argentatus* and *argenteus* are *shaded*. *Colors* in *pie charts* correspond to clade I (*green*) and clade II (*orange*) haplotypes in the mitochondrial network (see Fig. 4). The *black* portion in the Estonian population represents two individuals carrying a southerly distributed *cachinnans*-type sequence

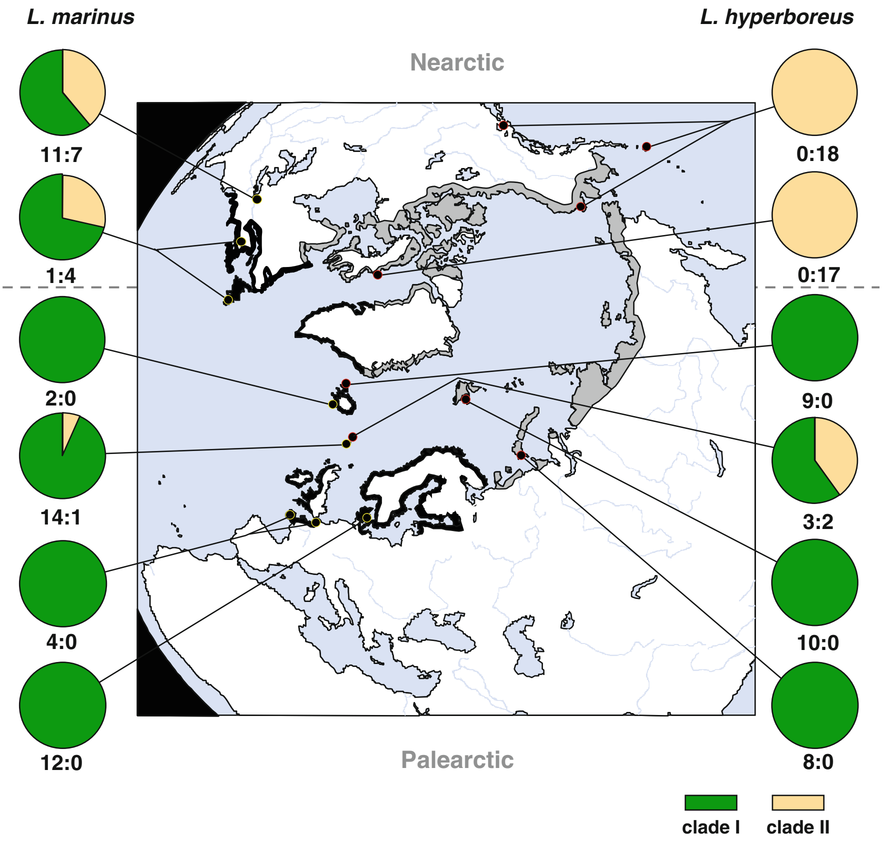

Fig. 9 Breeding ranges, sampling locations (*dots*) and haplotype composition (*pie charts*) of *L. marinus* ($n = 56$) and *L. hyperboreus* ($n = 67$). *Colors* in *pie charts* correspond to clade I (*green*) and clade II (*orange*) haplotypes in the mitochondrial network (see Fig. 4)

from the Faeroe Islands, carry clade I haplotypes (green portion in Fig. 9). Either the Nearctic or the Palearctic population acquired its mitochondrial haplotypes through hybridization – in North America with *smithsonianus*, or in Europe with *argentatus*.

Of the four European great black-backed gull *L. marinus* populations, all but one individual from the Faeroe Islands display clade I haplotypes (green portion in Fig. 9). Among North American *L. marinus*, clade I still predominates but clade II haplotypes account for a substantial amount of sequence variation.

We decided to use the amplified fragment length polymorphisms (AFLP) technique (Vos et al. 1995) because it allows the screening of a large number of autosomal loci and has proven to be very informative (Bensch and Akesson 2005).

Overall results: An extensive AFLP screening among seven large gull taxa yielded 17 primer combinations that could be scored with sufficient reliability. The resulting AFLP matrix consisted of the 1/0 scores of 230 loci among 369 individuals (Sternkopf et al., in preparation). We performed a principal component analysis (PCA), assuming within-group correlation. The plot of the first three principal components values, explaining 72.7% of the total data variation, is depicted in Fig. 10. This clearly demonstrates that five taxa, including *argentatus* and *marinus*, are genetically well defined and form distinct groups. The two remaining taxa, *smithsonianus* and *hyperboreus*, display partially overlapping clusters strongly suggesting a close autosomal genetic affinity.

3.2.1 Evolution of Herring Gulls in Europe

By means of AFLP loci, it was not possible to reproduce the strong biphyletic distribution among European herring gulls *L. argentatus* as was observed using mitochondrial sequences (Fig. 4). We were unable to detect significant differences in AFLP frequencies between herring gulls grouped according to mtDNA-defined clade I versus clade II (results not shown). Also, when we compared AFLP frequency patterns between northern and southern herring gull populations (see Fig. 8), no significant differences were found (results not shown). Even if we compare the two most extreme colonies with almost pure clade I mtDNA haplotypes from the White Sea and the almost pure clade II mtDNA haplotypes from the Netherlands, no significant AFLP distribution differences could be observed.

On the contrary, the PCA analysis (Fig. 10) and several Structure analysis runs (Pritchard et al. 2000; Falush et al. 2007) illustrate, that clade I and clade II herring gulls were not separable (results not shown), but formed a uniform cluster separate of the other six species (Fig. 10). Population differentiation and nuclear gene flow relationships also showed that *L. argentatus* from both mitochondrial clades were not significantly differentiated from each other, while they are from the other species (Sternkopf et al., in preparation).

These results confirm our prediction that clade I and clade II herring gulls form a single reproductive community. Their biphyly in the mitochondrial gene tree must, therefore, be the footprint of past gene flow between birds derived from different ancestral refugia.

This supports our original hypothesis (Liebers et al. 2004) that birds derived from the Aralo-Caspian refugium, possibly members of a pre-*heuglini* population, must have hybridized with the ancestral *argentatus* population from the North Atlantic. The introgressed clade II mitochondrial lineage persisted and has diversified to this day in the *argentatus* population.

3.2.2 Phylogeographic History of Circumpolar Breeding Glaucous Gulls

Among the circumpolar breeding glaucous gull L. *hyperboreus*, we see an even more distinct geographic distribution of mitochondrial haplotypes. All *hyperboreus* from Nearctic breeding colonies display exclusively clade II haplotypes, whereas Palearctic birds display clade I sequences (Fig. 9). The only two Palearctic

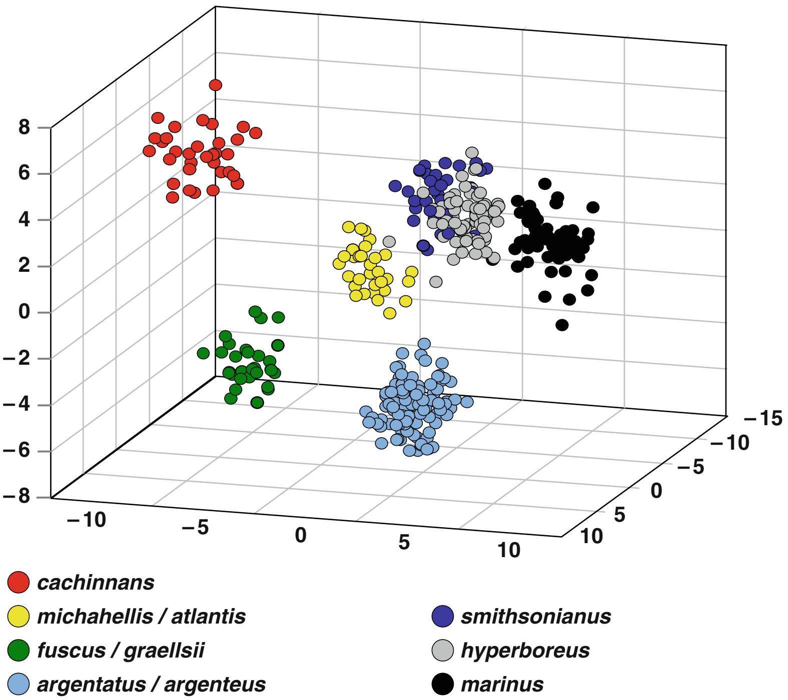

Fig. 10 Principal component analysis of total 1/0 AFLP data matrix ($n = 369$ individuals). The *axis* represents the first three principal component values

hyperboreus with clade II sequences were individuals wintering on the Faeroe Islands. The nuclear AFLP-markers do not co-segregate with the mitochondrial ones and show no differentiation of Nearctic versus Palearctic populations. On the contrary, Nearctic and Palearctic birds group together and overlap significantly with North American herring gulls (*L. smithsonianus*) indicating that both taxa are each other's closest relatives based on autosomal marker (Fig. 10).

Most probably, glaucous gull is an original member of the Beringian radiation (Fig. 4) that originated and diversified along the North Pacific/north-west Arctic coasts of North America and north-east of Russia. Only very recently, e.g., after the last deglaciation event of the Nearctic, could *hyperboreus* have spread into northern Europe where it came into contact with *argentatus* birds of clade I (see Fig. 8, green portion). Palearctic populations of *hyperboreus* must have acquired their mitochondrial haplotypes through hybridization with *argentatus* in Europe. So far, our Palearctic sampling reaches as far east as Taimyr. To fully resolve the circumpolar expansion, we should also include samples from north-eastern Siberia. Obviously, only with those samples can we reconstruct the complete population history and introgression process of *hyperboreus*.

Already at this stage of research, glaucous gulls constitute a textbook example of “mitochondrial take-over”, e.g., complete replacement of the original mitochondrial haplotypes (clade II) through hybridization with birds of the other clade.

3.2.3 Colonization Pattern of Greater Black-Backed Gulls

Nuclear markers confirmed the phylogenetic position of *marinus* within the herring gull complex (Fig. 7) contrary to the traditional view of great black-backed gull being a distinct outgroup, although *L. marinus* is fully reproductively isolated from all species with which it co-occurs (Liebers et al. 2004).

Based on mitochondrial sequences, *marinus* shows a biphyletic structure. Palearctic populations exclusively carry clade I, with a single exception on the Faeroe Islands, while Nearctic populations display a substantial proportion of clade II haplotypes (Fig. 9). Based on AFLP loci, it is not possible to reliably distinguish between North American and European *marinus* (Sternkopf et al., in preparation).

We suggest that *marinus* developed originally in northern Europe as a member of clade I. Only very recently, it colonized the coasts of eastern North America. The combined mitochondrial and AFLP results strongly suggest a brief period of hybridization in the Nearctic with members of the Beringian radiation, after which *marinus* rapidly became reproductively isolated again. This process involved only a few individuals and lasted only for a limited number of generations. Clade II mitochondrial haplotypes, that invaded the population via introgression, are now captured in the genepool of *marinus* and mask the true phylogenetic history of this species.

3.2.4 Summary

Mallet (2005) suggested that about 10% of animal species, mostly the younger ones, hybridize and hence continue to exchange genetic material through introgression. Many studies of closely related species reveal introgression, mostly of maternally inherited mitochondrial DNA. For example, in mammals, it was first discovered in house mice (Ferris et al. 1983; Prager et al. 1993), followed by a growing list of species, e.g., voles (Tegelström 1987), deer (Cathey et al. 1998), hares (Thulin et al. 1997; Melo-Ferreira et al. 2005), and chipmunks (Good et al. 2008). In birds, introgression through hybridization has been proven in *Hippolais* warblers (Secondi et al. 2006), *Vermivora* warblers (Vallender et al. 2007), and Caribbean banabaquit (Bellemain et al. 2008).

The consequences of such cryptic hybridization broadly echo and emphasize that extreme caution is needed when interpreting single gene genealogies, especially those based on mitochondrial DNA alone (Brito and Edwards 2008). The biphyletic representation of herring gulls, glaucous gulls and great black-backed gulls in the mitochondrial haplotype network provides a striking illustration of how discrepancies can arise between a gene tree (in this case, based on mitochondrial

DNA) and a taxon phylogeny (based on autosomal markers). The fact that some species, apparently due to past gene flow episodes, contain highly divergent mitochondrial haplotypes suggests that lineage sorting could have quite different and unpredictable outcomes (Gadagkar et al. 2005; Rokas and Carroll 2005).

In closely related species, such as the herring gull radiation, only a multilocus analysis of mitochondrial and nuclear markers can probably reveal the complex history of population subdivision and gene flow. Our results highlight the importance of using information from independent genetic markers when evaluating the evolutionary history of an adaptive radiation.

4 Future Perspectives

Despite the use of extensive mitochondrial DNA sequence variation, autosomal intron sequence variation, and a large number of independently segregating autosomal AFLP loci, we were unable to completely reconstruct the ancient demographic events resulting in the present day biphyletic position of three members of the large gull radiation: herring gull *L. argentatus*, glaucous gull *L. hyperboreus*, and great black-backed gull *L. marinus*. For a much better insight into the – probably relatively recent – events giving rise to the present day genetic variation observed in the herring gull radiation, much more autosomal DNA information is required. Here, we see two clearly distinct approaches. The first is the identification of a high number of genome-wide distributed autosomal co-dominant single nucleotide polymorphisms (SNPs) that could be used to reconstruct the genetic affinities among closely related groups of individuals. Such an approach can be applied successfully as has been clearly demonstrated by a number of studies using many thousands of SNPs among distantly and closely related human populations (Jakobsson et al. 2008; Lao et al. 2008; Li et al. 2008; Novembre et al. 2008). Another approach involves the use of relatively short but highly polymorphic non-recombining stretches of autosomal introns and/or exons that could serve as multiple mini-haplotype blocks. Such autosomal haplotypes, when sufficiently polymorphic, are ideal not only to retrace subtle gene flow processes among populations but will also facilitate the relative timing of such processes. For both approaches, there are now a growing number of biotechnological solutions, including extremely high-throughput sequencing (Vera et al. 2008), and the identification of conserved genome fragments by simply comparing the growing number of (nearly) completely sequenced whole genomes (Backström et al. 2008).

In addition, divergence levels at autosomal versus Z-chromosomal loci could be compared to test whether reinforcement played a significant role in the speciation process of large gulls. We hypothesize that differentiation has progressed further on the Z-chromosome than on the autosomes because Z-chromosomal loci are more likely to be related to, or linked to, loci causing reproductive incompatibilities (Servedio and Sætre 2003; Sætre et al. 2003; Borge et al. 2005; Mank et al. 2007; Qvarnström and Bailey 2009). Finally, contrasting genetic signatures from avian

W- and Z-chromosomes can be informative for inferring and reconstructing sex-specific gene flow patterns in large gulls.

To this end, we have already identified ~35,000 short autosomal genome fragments among four different members of the herring gull complex that could serve as the basis of further research using the approaches described above.

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